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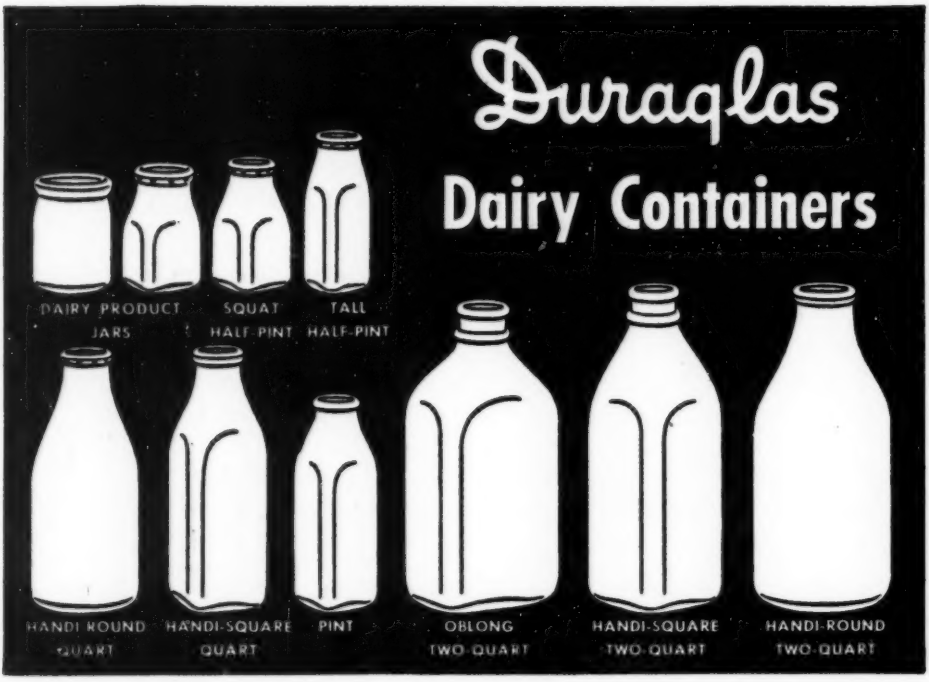

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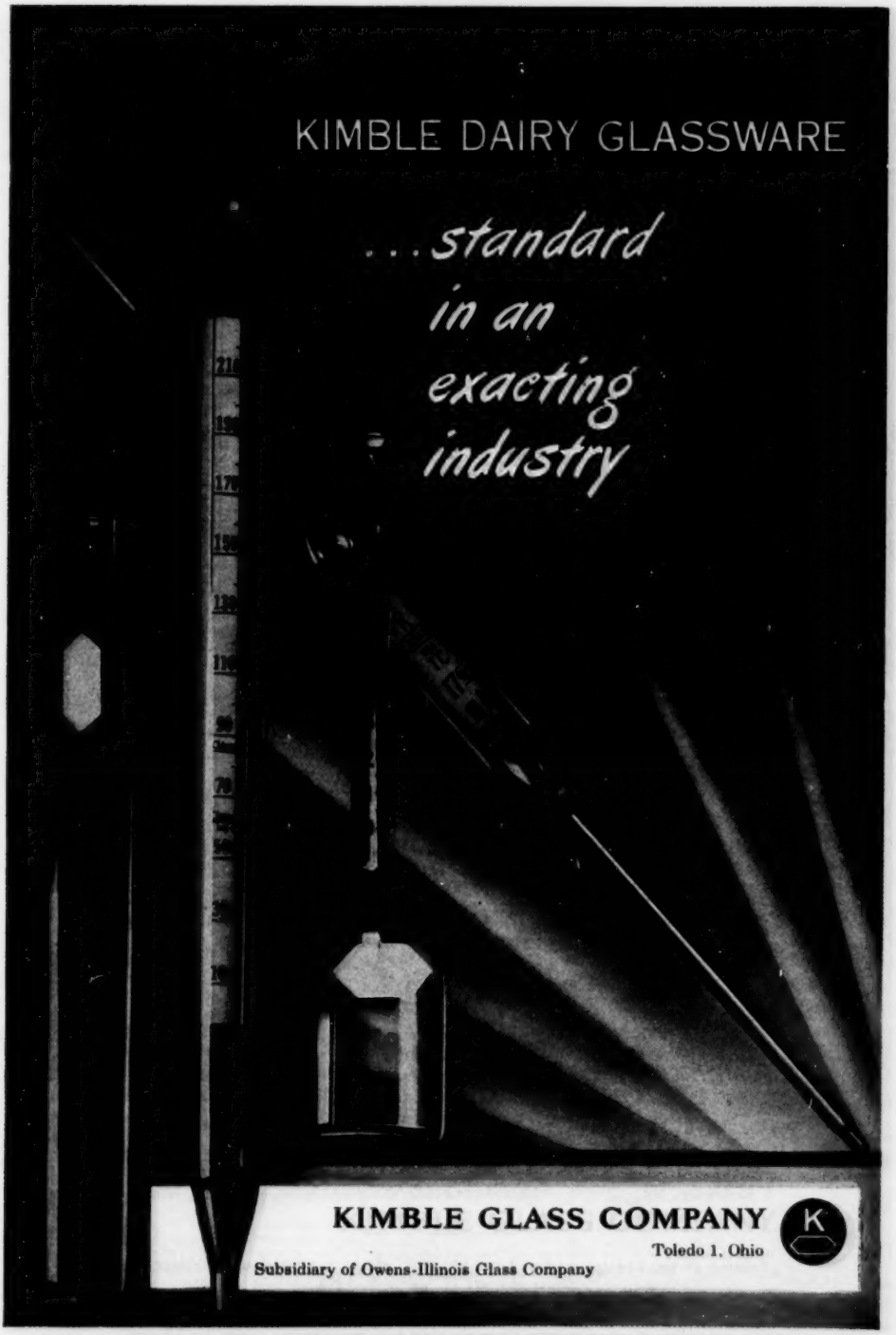

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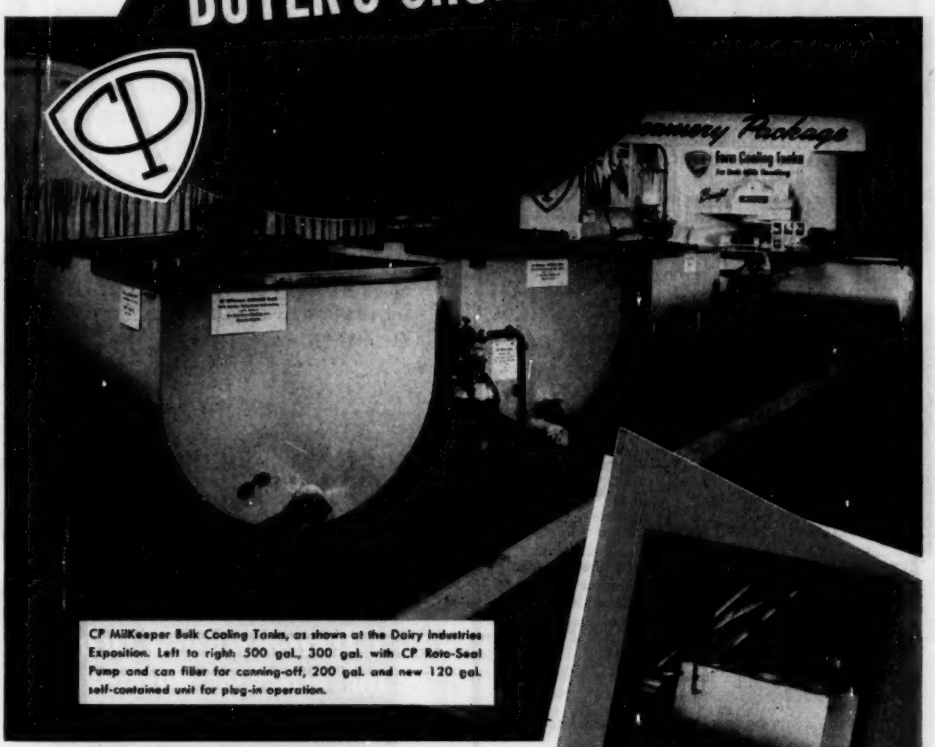

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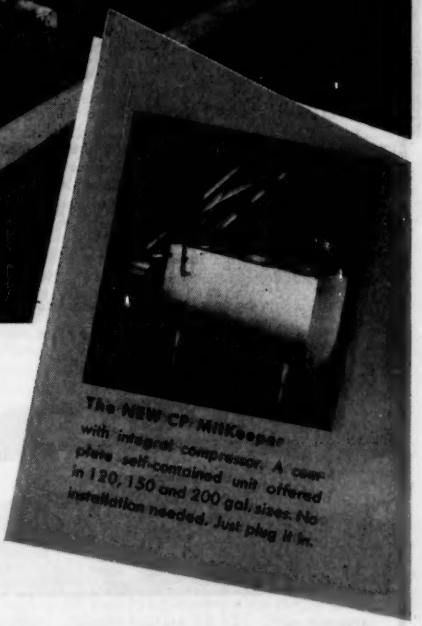

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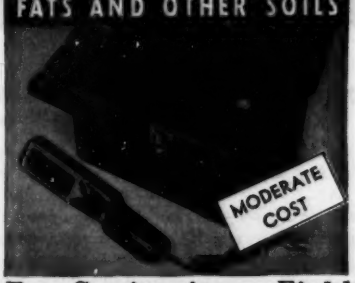

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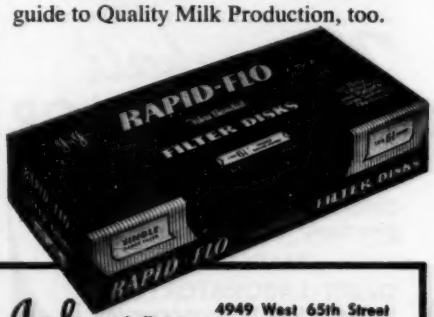

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JOURNAL OF DAIRY SCIENCE

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LACTATION STUDIES. III. VARIATIONS IN DAILY YIELDS OF DAIRY COWS[1, 2]

R. E. ERB, MARY M. GOODWIN, R. A. MORRISON[3] AND A. O. SHAW
Department of Dairy Science, The State College of Washington, Pullman

Most people associated with dairying realize there is a certain amount of daily variation in yields of dairy cows. The magnitude of these variations depends on management procedures (1, 2, 4, 5, 7, 10, 14, 15, 23, 24, 26), normal physiological changes in the cow (8, 9), anatomical defects of the udder and udder injuries. Individual cows vary in ease and completeness of milking (6, 11, 12, 13, 19, 20). These many factors influence the accuracy of lactation records and thus, indirectly, the reliability of the results for which these records are used.

While different types of management have a marked influence on variations in yields, it is not so easily understood why this variation should exist for those herds following all the currently recommended practices. Variations in butterfat test are a continual source of friction between dairymen and plant operators (10, 17) which shows no indication of diminishing even though considerable effort has been made to explain reasons for such discrepancies.

The purpose of this study was to determine sources and magnitude of variations in yields of cows under the same feeding and management practices throughout a lactation and milked two and three times daily.

PROCEDURE

Individual milkings of 19 Holsteins in the State College of Washington dairy herd (nine milked two times and ten milked three times daily) were weighed and tested for butterfat content for a 305-day period. Production records were started on the seventh day after parturition—referred to hereafter as the first day of lactation. The experimental period extended from October 22, 1947, through December 30, 1948. The cows were maintained in dry lot and received good quality alfalfa hay and either grass-alfalfa or pea vine silage. Grain was fed for near maximum production, but was not higher than 1 lb. for each 3 lb. of 4 per cent milk or above 18 lb. daily for the highest producing cows. The grain mixture contained approximately 14.5 per cent crude protein. Cows showing estrus

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[2] Acknowledgment is made to W. T. Southworth for assistance with I.B.M. analysis of data used for this study.

[3] Military leave of absence.

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were segregated. Milking intervals were equal, except when occasional power failures caused delay. Emergency power was rather promptly available in each instance. The cows were machine stripped and then checked by hand stripping to insure as nearly as possible complete milking. Two- and three-time milkings were started at 2:30 a.m. and 2:30 p.m., 4:00 a.m., noon and 8:00 p.m., respectively.

In addition, records were summarized for 15 cows milked and tested four times daily in various parts of the United States during the years 1914 to 1923 by official supervisors of the Holstein-Friesian Association. Milkings were at 6-hr. intervals starting between midnight and 4 a.m. Statistical procedures were as outlined by Snedecor (18) with all tests of significance reported herein based on methods of variance.

RESULTS AND DISCUSSION

Lactation curves were plotted for the three groups of cows under study. Since the curves for daily milk yields agree with those of Woodward (24), and the observations on fat test are similar to those of Weaver and Matthews (22) and Woodward (25) they are omitted from this report. Average daily milk yields increased during the first 40 to 60 days for all three groups of cows. Cows milked three and four times daily showed more persistency of fat yield during the first 80 days of lactation and greater persistency of milk yield during the latter part of the lactation than cows milked twice daily. Five of the cows milked three times daily carried calf 180 days or more as compared with four cows milked twice daily. This information was not available for the cows milked four times daily; however, since each cow had a 365-day record, it is doubtful if any carried calf more than 180 days during the first 305 days of their records. The average fat test declined very rapidly during the first 2 wk. on test. Since milk yield was increasing rapidly during the same period, the average yield of fat and 4 per cent fat-corrected-milk (F.C.M.) was highest for the first 2 wk. for cows milked two and three times daily. Cows milked four times daily showed increase in yield of F.C.M. during the first 3 wk. on test.

Variations between milkings. Average yields by milkings show that the cows milked three and four times daily gave, on the average, 0.5 and 0.8 lb. more milk, respectively, at the earliest morning milking as compared with the other milkings. Cows milked twice daily gave slightly less milk at the morning milking. This was due to one cow that frequently gave twice as much milk at the evening milking. These observations are in good agreement with Campbell (3, 4) who studied effect of hours of darkness. Average fat test was 0.09, 0.18 and 0.23 per cent lower at the earliest a.m. milking compared to the average of the other milking for cows milked, respectively, two, three and four times daily. This increase may be partially accounted for by exercise, since Woodward (23) noted increases in fat test when cows were forced to walk 3 miles. Exercise should have been minimal in this experiment (unknown for cows milked four times daily), since the cows spent a major portion of their time in the barn and no time grazing.

Variation by days in lactation. The standard error of estimate ($s_{y.x}$) was de-

termined for each of the 19 cows milked two and three times daily (table 1). Cow 243 was omitted from all statistical analyses because of intermittent attacks of ketosis for the first 5 mo. of the lactation. There was considerable difference between cows. There was no significant difference attributable to age or times milked daily for average daily variations in yield of fat and milk when corrected for average lactation decline. It is realized that $s_{y.x}$ is too high for milk yield, since the curve is not linear—particularly during early lactation. However, estimates of monthly yields are most generally based on one milk weight and one fat test per month. Since the initial supervised test can occur more or less at random during the first 2 to 6 wk. of lactation, the results of a linear correction would seem to have the more practical application. The $s_{y.x}$ for fat test

TABLE 1

Average yields and standard errors of estimate ($s_{y.x}$ on a cow per day basis) for 19 Holstein cows

Cow no.	305-day lactation yield							
	Times milked	Age	Milk	$s_{y.x}$	Fat	$s_{y.x}$	Fat	$s_{y.x}$
		(*yr.-mo.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(%)	(%)
243	2	6–5	15166	10.2	460.3	0.31	3.04	0.63
270	2	3–10	13207	4.1	449.9	0.21	3.41	0.51
284	2	2–6	14333	5.4	495.0	0.16	3.45	0.46
229	2	6–9	13461	3.1	503.4	0.05	3.75	0.35
283	2	2–5	10118	1.3	331.4	0.04	3.28	0.35
267	2	3–11	13236	3.8	484.3	0.17	3.66	0.37
285	2	2–6	10969	1.6	403.7	0.12	3.68	0.35
903	2	2–9	10710	3.9	393.2	0.16	3.67	0.50
245	2	6–0	14966	3.4	500.9	0.16	3.35	0.37
280	3	2–9	14345	3.4	479.9	0.03	3.35	0.21
207	3	8–2	18513	5.8	642.7	0.25	3.47	0.31
236	3	6–5	24430	4.0	802.7	0.23	3.28	0.27
274	3	3–2	19326	3.2	542.0	0.16	2.80	0.21
268	3	3–11	16368	3.9	564.5	0.15	3.45	0.33
251	3	5–9	20573	5.1	664.0	0.24	3.23	0.30
290	3	2–3	12321	2.6	477.1	0.18	3.87	0.29
288	3	2–5	13794	5.1	514.8	0.19	3.73	0.24
287	3	2–5	14206	3.2	454.2	0.12	3.20	0.27
278	3	3–0	14411	3.5	536.8	0.12	3.72	0.28

averaged 0.41 per cent per day for cows milked twice daily as compared with 0.27 per cent for cows milked three times daily. This difference was highly significant. When $s_{y.x}$ was expressed as a percentage of the average daily yield and subjected to analysis of variance, these cows showed no significant differences attributable to yield or age. It is realized these two factors are confounded, but the two combined accounted for only 1.5 per cent of the total variation. The average variation ($s_{y.x}$/daily mean) for milk, fat and percentage fat was 7.6, 9.0, 9.6 per cent, respectively. Average daily yield of milk was significantly less variable than fat or percentage fat. Level of production was divided into a lower half and upper half by times milked, in order to prevent confounding with times milked. The average variability was 8.6 per cent for the lower producers and 8.8 per cent for the higher producers. By this analysis, cows milked

TABLE 2

Average variation (s-b/mean daily yield) between cows milked 2- and 3-times daily

Times milked	No. of records	Component of yield			
		Milk	Fat	Per cent fat	Combined
		(%)	(%)	(%)	(%)
2	8	7.9	9.1	11.6	9.5
3	10	7.4	8.8	8.0	8.1
Total	18	7.6	9.0	9.6	8.7

twice daily varied significantly more than cows milked 3 times daily. This was predominantly due to the greater variation in fat test (table 2). This is confirmed by the earlier observation that milk and fat variations were non-significant, but the fat test was, between cows milked two and three times daily.

The foregoing analyses yielded valuable information relative to differences between times milked, age and level of production with respect to average daily variations around normal lactation decline curves. However, it does not tell the whole story. D.H.I.A. and Herd Improvement Registry (H.I.R.) methods use 1-day milk weights and one test per month. The question is whether some stages of lactation yield more error in estimation of yields than do other stages. To answer this question, the standard deviation was determined for each of the ten monthly test periods for each cow, using daily yields for each calendar month. The limits of one, two and three standard deviations from the mean for milk, fat and percentage fat are shown in figures 1, 2 and 3. The data on charts are shown

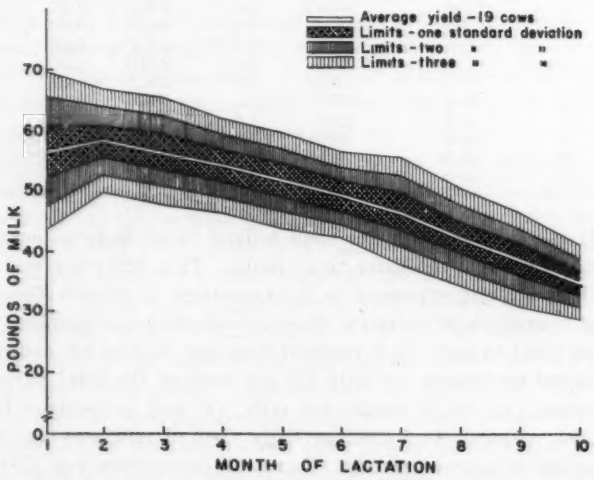

FIG. 1. Limits of one, two and three standard deviations from the mean milk yields of 19 cows. Daily yields by individual cows by month of lactation were used for the original calculations.

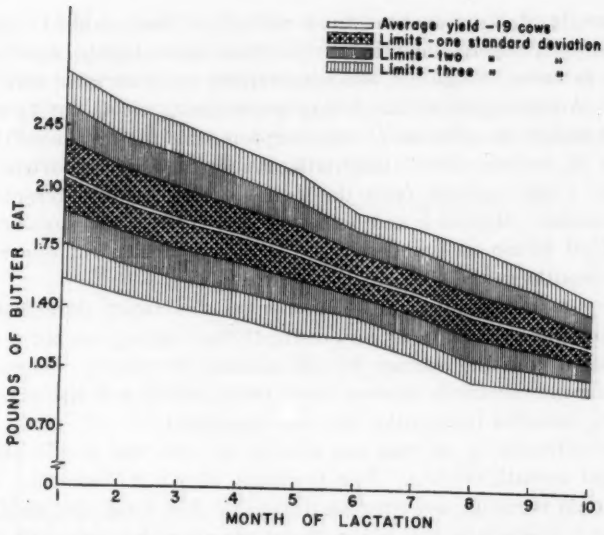

FIG. 2. Limits of one, two and three standard deviations from the mean fat yield of 19 cows. Daily yields by months of lactation were used for the original calculations.

proportionately so that direct comparisons can be made for milk, fat and percentage fat with respect to expected variability for each month of lactation. Analysis of variance of these data was made in two ways as previously. First, separate analyses were made for milk, fat and percentage fat. Then these were combined by expressing each as a percentage of the respective monthly means on a per cow per day basis. This permitted a test of significance for components of variation. Daily variations of milk yield within months were not significant

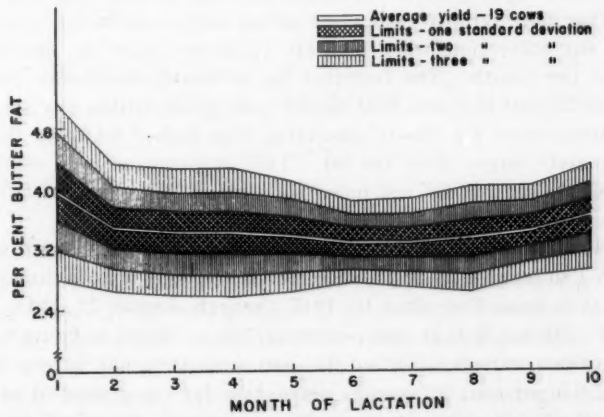

FIG. 3. Limits of one, two and three standard deviations from the mean butterfat test of 19 cows. Daily yields by months of lactation were used for the original calculations.

between cows, level of production, times milked, or times milked × month interaction. Daily variations × months in lactation were highly significant. The first month, as shown in figure 1, was considerably more variable than any of the other nine. A major part of this is due to increasing yields during early lactation and represents an estimate of error expected for the first month's yield for those forms of testing where daily milk weights are not reported. However when month 1 was omitted from the analysis, significant differences between months remained. Months 5 and 6 showed significantly less variation than the average for all 10 mo. as based on least significant mean differences using the mean of all months as the basis for the comparisons.

Average variation in fat yield within months of lactation showed significantly more variation for the first 3 mo. and distinctly less during the last 5 mo. (figure 2) as compared with the average for all months ($P < 0.01$). There also were highly significant variations between cows, times milked and age of cow. Times milked daily × months interaction was non-significant.

Average variation in fat test was greatest for the first month and least for the sixth and seventh months. This is clearly shown in figure 3. In general, month-to-month variation was greatest during the first 5 mo. and smallest during the last 5 mo. There were highly significant variations between cows and months of lactation and significant variations between two- and three-time milking. There were no significant variations attributable to times milked daily × month interaction or to age or yield.

Expressing standard deviation as a percentage of the daily mean showed that milk was less variable than fat for each of the 10 mo. and was less variable than fat test for all months except 8, 9 and 10. The variations were highly significant, with fat yield being the most variable. The results of these studies show rather clearly that if investigators are going to use partial lactation records of 150 to 180 days in length to avoid late lactation disturbances, they also ought to omit the first 30 to 50 days of the lactation for the same reason. In fact, these data emphasize that more error of estimation occurs early in lactation, *i.e.*, the first 30 days, than any other time when monthly yields are based on one milk weight and one test per month. The fact that fat is considerably more variable than milk yield or fat test indicates that higher milk yields within any small segment of the lactation curve are closely associated with higher fat tests, thus yielding a proportionately larger error for fat. This phenomenon is in addition to the well established fact that fat test increases as the normal yield of milk decreases as lactation progresses.

Day-to-day variation. The nine cows milked twice daily were all in milk from March 1 to August 23, 1948, whereas the ten cows milked three times daily were all in milk from December 10, 1947, through August 22, 1948. The daily averages for milk, fat, F.C.M. and percentage fat, as shown in figure 4, clearly indicates the rather extreme variation that can occur from one day to the next. A variation of 0.5 per cent in average percentage fat for groups of nine and ten cows, respectively, is quite common. The extremes approach 1 per cent from one day to the next. Thus, the hazards of attempting to compare average tests

observed by a D.H.I.A. tester and what is observed at the plant are clearly demonstrated. These data do not include cows recently fresh or cows drying off as is the case where comparisons between tests at the farm and at the plant are made over a period of time. Neither is there a possibility that some milk is being taken for calf feeding or household use. In other words, this is a demonstration of day-to-day variability at its source.

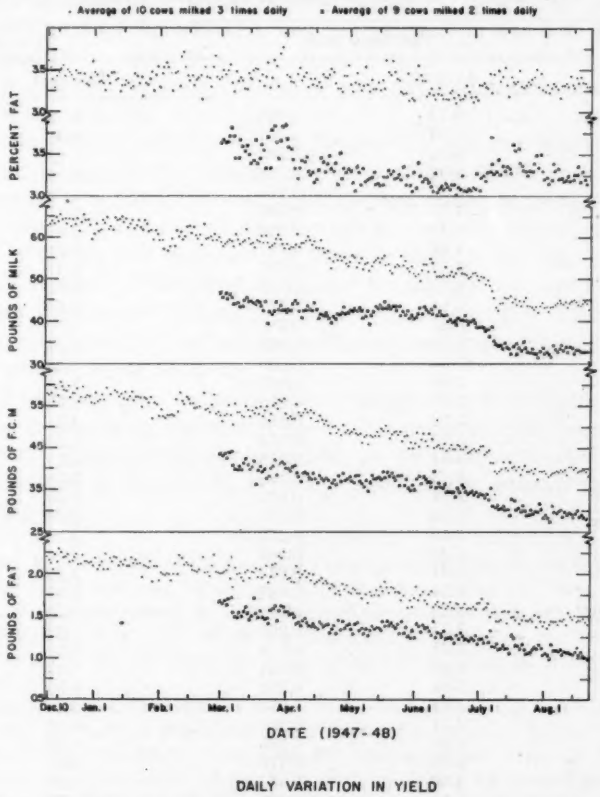

FIG. 4. Average daily variation in yield by days of the year for cows milked two and three times daily. (Cows in different stages of lactation.)

Error in estimating butterfat test. It is known that fat test of Holstein cows is higher early in lactation and that it declines rather rapidly during the first 30 to 40 days. Days were selected throughout the lactation to give some comparative measure of daily variation from one part to the other. These data, as shown in table 3, reveal that one could hope to estimate fat test with $\pm$ 0.8 per cent during the second week of lactation (days 1–7) about two-thirds of the time. This day-to-day variability becomes more constant after 10 days for cows milked three times daily, but is quite variable yet at 20 days for cows milked

twice daily. One might question the advisability of testing cows as early as the seventh day after freshening, as was the case with these cows. It is realized that pre-partum milking for varying lengths would have an effect, but this is not a common management practice. Actually, a cow could gain advantage or

TABLE 3

Variations in fat test by day of lactation for cows milked twice and three times daily. (No adjustment made for inherent differences between cows)

Day of lactation	Two times daily		Three times daily	
	Av. test	s	Av. test	s
1	5.11	1.025	4.16	0.900
2	5.08	0.877	4.06	1.110
3	5.04	0.860	3.88	0.700
4	4.91	0.748	3.79	0.600
5	4.77	0.794	3.76	0.707
6	4.79	0.640	3.75	0.671
7	4.49	0.656	3.67	0.600
8	4.24	0.707	3.54	0.592
9	4.63	0.755	3.41	0.500
10	4.70	0.735	3.62	0.480
15	3.84	0.721	3.50	0.435
16	3.81	0.980	3.50	0.497
17	3.79	0.949	3.54	0.446
18	3.59	0.609	3.55	0.495
19	3.66	0.872	3.42	0.454
20	3.78	0.686	3.37	0.423
50	3.72	0.480	3.31	0.311
51	3.63	0.374	3.32	0.422
52	3.44	0.332	3.31	0.457
53	3.43	0.155	3.38	0.421
54	3.44	0.243	3.29	0.410
55	3.58	0.255	3.36	0.470
150	3.40	0.272	3.34	0.343
151	3.36	0.202	3.46	0.365
152	3.41	0.274	3.32	0.243
153	3.34	0.161	3.46	0.243
154	3.23	0.297	3.39	0.327
155	3.31	0.266	3.40	0.362
200	3.38	0.170	3.23	0.345
201	3.26	0.141	3.25	0.276
202	3.24	0.232	3.30	0.389
203	3.28	0.255	3.22	0.341
204	3.34	0.338	3.22	0.288
205	3.35	0.266	3.36	0.302
250	3.36	0.170	3.25	0.386
251	3.50	0.167	3.39	0.424
252	3.54	0.167	3.28	0.345
253	3.62	0.158	3.39	0.390
254	3.70	0.170	3.32	0.338
255	3.48	0.173	3.39	0.362

disadvantage with equal opportunity. Degree of edema at freshening and other complications also could alter the initial yields. Perhaps to increase uniformity, cows should not be tested sooner than 10 to 14 days after freshening.

SUMMARY

Individual milkings of 34 Holstein cows were weighed and tested for butterfat content throughout a 305-day lactation period. Nine of these cows were milked

twice daily and 10 were milked three times daily under similar conditions of management and environment. Average milk yield was higher and fat test was lower for the early morning milkings as compared with those later in the day.

Using standard error of estimate ($s_{y.x}$), no significant differences were found in daily variation in yield of milk and fat between cows milked twice and three times daily. The difference in daily variation of fat test was highly significant, with cows milked twice daily being the most variable. The average daily variation ($s_{y.x}$/daily mean) for milk, fat and percentage fat was 7.6, 9.0 and 9.6 per cent, respectively. Milk yield was significantly less variable than fat yield or percentage fat. These daily variations were not significantly influenced by differences in age or yield.

The relative accuracy of milk weights for one day and one butterfat test per month for calculating monthly yields was measured for individual cows. Month-to-month variations in yield were maximum the first month in lactation and proportionately less each succeeding month until the seventh month of lactation. Each of the 10 mo. showed less variation for milk than for fat yield. Butterfat test likewise was more variable than milk yield for the first 7 mo. of lactation.

Day-to-day variations of 0.5 per cent in average butterfat test for groups of nine and ten cows were not uncommon, with extremes approaching as much as 1 per cent from one day to the next.

Errors in estimating butterfat test based on one test per month were more than twice as great during the second week after parturition as compared with the ninth week. This observation would lead one to question the advisability of testing cows as soon as the seventh day after freshening to estimate the yield for the first testing period.

REFERENCES

(1) Anderson, A. C. Studies in the Range of Variation of the Per cent of Butterfat in the Milk of Individual Cows. Mich. Agr. Expt. Sta. Special Bull. 71. 1914.

(2) Blosser, T. H., and Smith, V. R. A Comparison of Total Milk and Fat Production and Per cent Fat of a 26- and 24-hour Test Day. J. Dairy Sci., 30: 951–956. 1947.

(3) Campbell, K. W. D. The Effect of Night on Milk Production. J. Dairy Research, 3: 52–60. 1931.

(4) Campbell, K. W. D. The Effect of Nine-, Twelve- and Fifteen-hour Intervals during the Day and the Night on Milk Production. J. Dairy Research, 4: 28–36. 1932.

(5) Davidson, F. A. The Effect of an Incomplete Removal of Milk from the Udder on the Quantity and Composition of the Milk Produced during the Immediate Subsequent Milkings. J. Dairy Sci., 7: 267–293. 1924.

(6) Dodd, F. H., and Foot, A. S. Residual Milk. Agr., 55: 238–242. 1948.

(7) Eckles, C. H., and Shaw, R. H. The Influence of Stage of Lactation on the Composition and Properties of Milk. U.S.D.A. B.A.I. Bull. 155. 1913.

(8) Erb, R. E., Goodwin, Mary M., Morrison, R. A., and Shaw, A. O. Lactation Studies. I. Effect of Gestation. J. Dairy Sci., 35: 224–233. 1952.

(9) Erb, R. E., Goodwin, Mary N., Morrison, R. A., and Shaw, A. O. Lactation Studies. II. Effect of Estrus. J. Dairy Sci., 35: 234–244. 1952.

(10) Gould, I. A., and Stout, R. E. Fat Variations in Milk. Part one—Farm Factors. Milk Plant Monthly, 38: 1–11. Mar., 1949.

(11) Johansson, I. Variations in the Fat Content of Milk Caused by Varying Degrees of Udder Evacuaton during Milking. XIIth Int. Dairy Congr., Stockholm, pp. 171–181. 1949.

(12) KNODT, C. B., AND PETERSEN, W. E. The Effect of Continuous Injections of Pitocin upon Milk and Milk Fat Production. J. Dairy Sci., **25**: 709–710. 1942.

(13) KNODT, C. B., AND PETERSEN, W. E. The Effect of Complete Evacuation of the Mammary Gland of Pitocin upon Milk and Fat Production. J. Dairy Sci., **27**: 449–457. 1944.

(14) MILLER, K., AND PETERSEN, W. E. Some Factors Involved in Efficient Milking. J. Dairy Sci., **24**: 225–233. 1941.

(15) MISNER, E. G. Economic Study of the Dairy-Herd-Improvement-Association Two-successive-milkings Tests compared with Monthly Average Milk Plant Tests. 100 New York Farms, September, 1947, through August, 1948. (Mimeo. Report) N. Y. St. Coll. Agr., A.E. 739 (1950). (Dairy Sci. Abs., **13**: 413. Dec., 1951.)

(16) RAGSDALE, A. C., BRODY, S., AND TURNER, C. W. The Variation in the Per cent of Fat in Successive Portions of Cow's Milk. J. Dairy Sci., **4**: 448–450. 1921.

(17) RAGSDALE, A. C., AND TURNER, C. W. The Stage of Lactation as a Factor in the Variation of the Per cent of Fat in Cow's Milk. J. Dairy Sci., **5**: 22–26. 1922.

(18) SNEDECOR, G. W. *Statistical Methods Applied to Experiments in Agriculture and Biology.* The Iowa State College Press, Inc., Ames. 1946.

(19) SWANSON, E. W., AND HINTON, S. A. Residual Milk from Oxytocin Injections throughout the Lactation. J. Dairy Sci., **34**: 419–426. 1951.

(20) SHAW, J. C. The Effect of Oxytocin on Milk and Milk Fat Secretion. J. Dairy Sci., **25**: 1051–1055. 1942.

(21) VOLCANI, R. Butterfat Content of Cow's Milk. A. Seasonal Fluctuation in the Butterfat Content. B. The Effect of Feeding Whole Citrus Fruit and Some of Its Parts on the Fat Per Cent. Bull. Rehovoth (Israel) Agr. Research Sta. no. 53. 82 pp. (Hebrew with English summary.) From abstract in Nutr. Abs. Revs., **20**: 6057. 1941. Animal Breeding Abs. (Edinburgh), **19**, 1134: 325. 1951.

(22) WEAVER, E., AND MATTHEWS, C. C. The Influence of Temperatures and Certain Other Factors upon the Percentage of Fat in Milk. Iowa Agr. Expt. Sta. Research Bull. 107. 1928.

(23) WOODWARD, T. E. Factors Influencing Percentage of Fat in Milk. J. Dairy Sci., **6**: 466–478. 1923.

(24) WOODWARD, T. E. The Production of Dairy Cows as Affected by Frequency and Regularity of Milking and Feeding. U.S.D.A.B.D.I. Circ. 180. 1931.

(25) WOODWARD, T. E. Some Studies of Lactation Records. J. Dairy Sci., **23**: 209–218. Mar., 1945.

(26) WOODWARD, T. E., HOTIS, R. P., AND GRAVES, R. R. Incomplete Milking in Relation to Milk Production and Udder Troubles in Dairy Cows. U.S.D.A.B.D.I. Tech. Bull. 522. 1936.

LACTATION STUDIES. IV. ACCURACY OF DIFFERENT METHODS OF ESTIMATING LACTATION YIELDS[1, 2]

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Previous papers in this series have described effects of gestation (6), estrus (7) and other causes of daily variation (8) in yields of dairy cows. These studies were felt necessary as a prelude to studying variations in estimating lactation yields by various methods.

Accepted methods for recording yields of cows have gradually changed over the years. The 7-day test was shown by Yapp (17) to be a poor indicator, while Gaines (9) later showed that 7-day tests conducted after 60 days in lactation were more indicative as an estimate of lactation yields. M'Candlish and M'Vicar (12) found that a 1-day test per month yielded results within 2 per cent of actual yield, and Dick (5) observed an average error of 2.32 per cent from actual when cows were tested at 28-day intervals. Houston (11) found that weekly test intervals gave estimated yields approximating actual, and that to keep errors within a range of 10 per cent the testing interval should not exceed 30 days. McDowell (13) found that monthly and bi-monthly tests varied from actual an average of 2.91 and 3.80 per cent, respectively. Gifford (10), Copeland (4), Bayley *et al.* (2), McKellip and Seath (14) and Alexander and Yapp (1) have presented data which indicate that bi-monthly testing adequately estimates lactation yield. Alexander and Yapp (1) also reported that testing the second, sixth and tenth; the second and tenth; and the fourth and eighth months of lactation gave sufficiently accurate results to merit consideration as a means of reducing the cost of testing. However, to follow these suggested programs of testing would require at least bi-monthly visits by the supervisor. Bayley *et al.* (2) found the average percentage error in milk yield was 3.0 and 5.0 for bi-monthly and quarterly testing, respectively, as compared with monthly tests. Fat yield varied 4.0 and 6.0 per cent for the same comparisons.

Cannon *et al.* (3) and Gaines (9) observed a high correlation between testing during the fifth month of lactation and monthly testing. McKellip and Seath (14), comparing the centering date and calendar month methods, found little difference in accuracy. Morrison (15), using eight different testing dates for each month, found a significant variation from actual when the calender month method was used, but none for the centering date method. The extremes for the centering date method were evenly distributed for the eight test days studied, which indicated it was by chance what dates happened to be high or low. The

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[3] On military leave of absence.

calendar month method always gave estimated yields above actual when the test dates were early in the month and below actual when test dates were at the end of the month.

From results of previous investigations, it appears that less costly means of testing are feasible. It was the purpose of this study to determine the deviations from actual yield of dairy cows for different methods of testing.

PROCEDURE

Yields of 19 Holstein cows in the State College of Washington dairy herd were recorded for the first 10 mo. of lactation. Ten were milked three times daily and nine were milked twice daily. Records of fat and milk yield were kept by milkings for a 305-day period starting with the seventh postpartum day, which hereafter will be referred to as day one of lactation. Calculations were made for nine cows milked twice daily to compare relative accuracy of testing at regular intervals of 30, 60, 90, 120 and 150 days, for 24- to 96-hr. periods with the first test centered on the cows' freshening date. In addition, the calendar month method was used for the monthly test intervals, assuming the tester could have first tested the cow on any day of lactation from 1 through 40 (as frequently happens on H.I.R.) and at monthly intervals thereafter. This gave 40 different calculations for each of nine cows. Total calculations for each cow for the centered methods were 31 for monthly, 62 for bi-monthly, 93 for every third month, 124 for every fourth month and 155 for every fifth month. For example, assuming a cow first could be tested on March 15 with the tester having equal opportunity of first testing the cow anytime during a period of 1 mo., there would be a total of 31 combinations. Testing intervals after the first test were on the same day of each succeeding month as the day of the initial test for each of the 31 possible combinations. This same procedure was followed for the longer intervals of testing.

The comparative accuracy of calendar month and centering date was calculated from average daily yields by day of lactation for nine cows milked twice daily, ten cows milked three times daily, and for a cow with average daily variation which was milked twice daily (8). Since a cow could first be tested on any day of the month, and all cows have equal opportunity to be eligible for the initial test on any day of the month, this yields 961 possible testing combinations for the calendar month method resulting from variable freshening within a month and variable days within the same month a tester might appear.

Comparison of current Herd Improvement Registry (H.I.R.) and Advanced Registry (A.R.) methods was made by selecting at random testing dates for each month of the year from the first, middle and last half of the month to approximate what happens when herds also are testing under Dairy Herd Improvement Association (D.H.I.A.) (centering method) procedures. A fourth series of dates was selected at random from the entire month to approximate what frequently is the case for herds not doing D.H.I.A. testing. Statistical procedures followed were as outlined by Snedecor (16).

RESULTS AND DISCUSSION

Centering date versus calendar month. The results of this study show that the calendar month method is approximately twice as variable as the centering date method (table 1). The main difference between the two methods is that it is possible for a cow to get anywhere from 7- to 68-days credit on the first test from the calendar month, and to get 21- to 51-days credit for the centering date. Cows first tested at the time they reach maximum milk yield will be overcredited by both methods. The overcrediting becomes maximum if the cows do not reach maximum production until 60 days in lactation, as was the case for this group of cows. Since it is possible to get 17 days more credit on initial test for the calendar month method, the possible error in estimation is proportionately greater. Since test drops rapidly (8) and reaches minimum at the time of maximum milk yield, fat yield is underestimated while milk is being overestimated. Cows tested early in the month to coincide with centering dates gain a distinct advantage when the same test dates are used for calendar month calculations. The reverse is true when test dates are near the end of the month. A third factor introducing differences between the two methods is the last testing period, where days of credit can on occasion be 15 days longer for the calendar month method. This causes no particular advantage when tested early in the month, but if a herd owner anticipates the tester and dries his cow off a day or two in advance, further error could be induced. For the method of calculation used in this study, the time first tested exerted the major influence. The errors by either method are negligible, but a standard method is indicated in the interests of uniformity when production credits are being calculated on the same cow using the same testing days.

Calculations made on daily averages of nine and ten cows, respectively, compare the two methods with respect to herd averages or to averages of a like number of daughters of a bull. Cow 267, showing average daily variation for the group of 19 cows (8), is presented as a typical example (table 1). The centering date method shows only one-half as much variation as the calendar month method. While the limits shown would be less for cows with less daily variation and more with highly variable cows, the proportional relationship between the two methods would be approximately the same.

Comparison of testing intervals. The centering method, based on day of freshening, was used to compare 30-, 60-, 90-, 120- and 150-day intervals of testing. The percentage error exceeded one time in four in estimating milk yields of individual cows was 2.4, 3.4, 5.6, 7.4 and 8.8 for the 30- to 150-day intervals of testing, respectively. The error in estimating fat yield was 1.2 to 2.5 per cent greater than for milk yield. The comparisons for 30- and 60-day intervals to actual are in good agreement with McDowell (13) and M'Candlish and M'Vicar (12). The comparison of testing every 90 days is in reasonable agreement with Bayley *et al.* (2) who used D.H.I.A. records for the error estimates. The results are not comparable with those of Alexander and Yapp (1), since they did not use all possible combinations of testing dates.

TABLE 1

Centering date versus calendar month for estimating lactation yields

Method	Milk	Fat	F.C.M.	%	Milk	Fat	F.C.M.	%	Milk	Fat	F.C.M.	%
	Average for 9 cows milked 2 times daily				Average for 10 cows milked 3 times daily				Cow 267			
305-d. actual yield (*lb.*)	12,906	447.0	11,867	3.46	16,829	567.9	15,250	3.37	13,236	484.3	12,559	3.66
Calendar month—percentage error exceeded 1 time in 4	1.9	2.8	2.3	1.9	1.5	1.7	1.5	1.2	4.6	6.1	5.2	3.4
Calendar month—percentage error exceeded 1 time in 100	4.2	6.2	5.2	4.3	3.3	3.8	3.4	2.8	10.3	13.7	11.6	7.5
Centering date—percentage error exceeded 1 time in 4	0.7	1.1	0.8	1.1	0.6	1.0	0.7	1.0	2.3	3.2	2.4	3.3
Centering date—percentage error exceeded 1 time in 100	1.6	2.5	1.7	2.5	1.3	2.2	1.5	2.3	5.2	7.3	5.4	7.5

TABLE 2

Percentage error of different testing intervals based on average deviations of nine cows milked twice daily

Average of 305-d. lactation yields for 9 cows		Interval of testing								
		30 d. (31)[a]			60 d. (62)[a]			90 d. (93)[a]		
		Milk	Fat	%	Milk	Fat	%	Milk	Fat	%
Actual yields (*lb.*)		12,906	447.0	3.46	12,906	447.0	3.46	12,906	447.0	3.46
Observed yields (*lb.*)		12,948	446.5	3.45	12,999	445.4	3.43	13,044	445.1	3.41
Percentage error exceeded 1 time in 4		2.4	3.7	3.5	3.4	5.4	5.4	5.6	6.8	6.6
Percentage error exceeded 1 time in 100		5.3	8.2	7.7	7.6	12.0	12.1	12.5	15.2	14.7
Reduction in percentage error for										
(Compared with 24-hr. test)	a) 48-hr. test	14.3	25.9	23.1	10.0	19.6	23.5	5.6	19.4	18.7
	b) 72-hr. test	20.7	36.4	31.7	15.7	27.8	29.0	8.6	27.8	25.8
	c) 96-hr. test	26.0	42.7	38.5	19.9	32.1	32.7	10.4	33.1	30.8
Percentage error exceeded 1 time in 4 on 96-hr. test		1.8	2.1	2.1	2.7	3.6	3.6	5.0	4.5	4.6
Reduction in error		−0.6	−1.6	−1.4	−0.7	−1.8	−1.8	−0.6	−1.3	−2.0
		120 d. (124)[a]			150 d. (155)[a]					
Observed yields (*lb.*)		13,070	444.7	3.41	13,107	444.4	3.39			
Percentage error exceeded 1 time in 4		7.4	8.7	8.5	8.8	11.3	8.8			
Percentage error exceeded 1 time in 100		12.4	19.6	19.0	19.8	25.2	19.6			

[a] No. of testing combinations for each cow.

The effect of testing 48, 72 and 96 hr. at each test interval was studied for the 30-, 60- and 90-day intervals of testing (table 2). The reduction in error based on actual yield was small, ranging from 0.7 per cent for milk to 1.8 per cent for fat. Increasing the length of the supervisory period for any interval of testing would not seem economically feasible.

The increase in error with length of testing interval is closely related to time the cow is first tested after freshening. This is rather clearly demonstrated in figures 1, 2 and 3 for the 60-, 90-, 120- and 150-day intervals of testing. Estima-

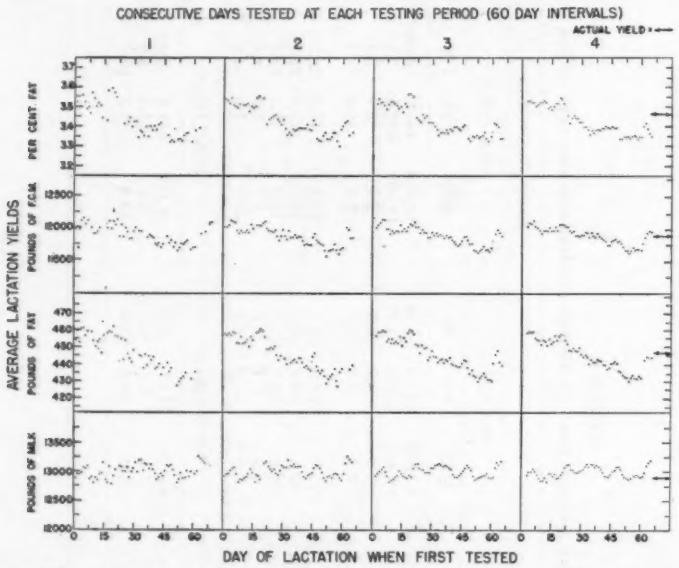

FIG. 1. Effect of time first tested and days of continuous supervision on accuracy of 60-day testing intervals.

tion of milk yield is not grossly affected by 60-day intervals. However, fat yield is, on the average, overestimated when the cow is first tested early in lactation and underestimated when the cow is first tested toward the end of the second month of lactation (fig. 1). Milk yield is underestimated for the 90-day interval when first tested anytime during the first month of lactation (fig. 2). This changes abruptly to overestimation if the first test occurs during the second month. This gives a testing pattern of the second, fifth, and eighth months of lactation and changes the number of supervisions from four to three times per 305-day lactation.

Both milk and fat yields are overestimated for the 150-day testing intervals when the first test occurs during the first 2 mo. of lactation. Both are underestimated when the first test occurs during the fourth or fifth month of lactation (fig. 3). Milk yield is overestimated for the 120-day test intervals when the cow first is tested between 60 and 90 days in lactation (fig. 3). The accuracy of

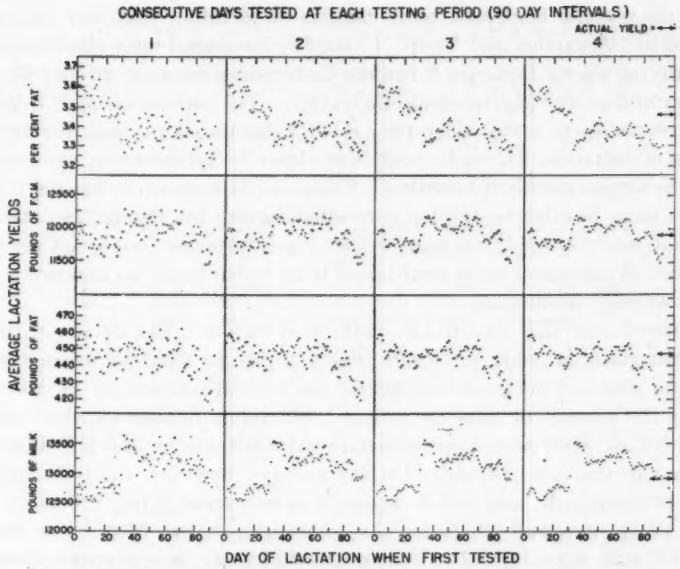

FIG. 2. Effect of time first tested and days of continuous supervision on accuracy of 90 day testing intervals.

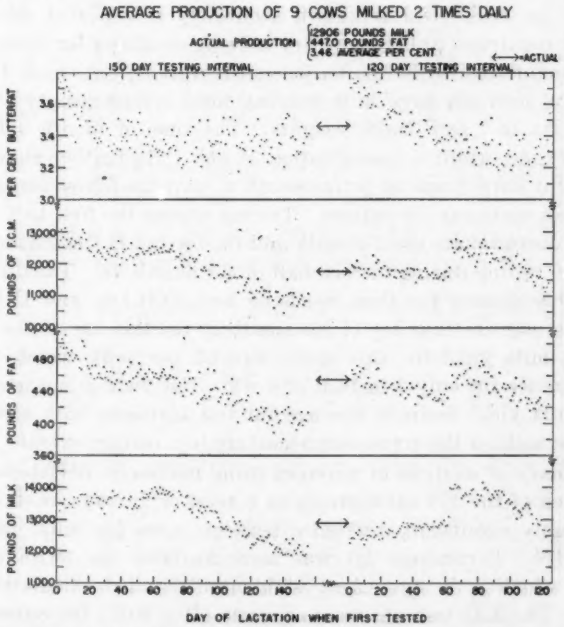

FIG. 3. Effect of time first tested and days of continuous supervision on accuracy of 120- and 150-day testing intervals.

testing the second, sixth and tenth months of lactation (120-day intervals) as reported by Alexander and Yapp (1) may be compared with observations from this study as shown in figure 3 for the first testing between 30 and 60 days of lactation and at 120-day intervals thereafter. The estimation of F.C.M. was in reality very close to actual when the cow first was tested anytime during the first 60 days of lactation, but milk yield was closer to actual when cows were first tested the second month of lactation. From the data shown in figures 1, 2 and 3, it would seem possible to develop correction factors for the various testing patterns that would occur if herds were tested only three or four times per year, so that fewer daughters of bulls would need to be tested to get an accurate appraisal of their average production.

Comparison of A.R. and H.I.R. methods of testing. The details of this study are summarized in table 3. There was a three- to five-fold greater range in maximum plus and minus deviations for the 115 calculations on 19 cows as compared to the average of these deviations. Maximum deviations when estimating fat and F.C.M. yield were approximately twice greater for the H.I.R. method as compared to the A.R. method. On the average, however, the difference in accuracy between A.R. and H.I.R. methods is not great being 120 lb. of F.C.M. for the 24-hr. test, and 99 lb. for the 48-hr. test, or less than 1 per cent when compared with actual yield. The two methods can be compared directly for estimation of fat yield where the differences are even smaller—2.2 and 1.7 lb., respectively, for the 24- and 48-hr. tests. The merit of the A.R. test seems greatest because milk yield is known accurately if reported accurately; and, since the test requires a preliminary dry milking, it allows for closer supervision. The latter would seem to be the major consideration; but, since the supervisor records several previous days' milk weights, there is opportunity to suspect herd owners who try to "pad" milk weights. The time of month a herd is tested seems to be as important a consideration as any. As further shown in table 3, selecting testing dates from the entire month *A*, gave the lowest average deviations but the highest maximum deviations. Testing during the first half of the month, *B*, tended to overestimate yield of milk and fat for the H.I.R. method and underestimate when testing during the last half of the month, *D*. The difference would be considerably greater for those herds on both D.H.I.A. and H.I.R. when the centering date was the first day of the month or the last day of the month. The difference in milk yield for this study was 1.2 per cent, which represents an average difference for only 2 instead of 4 wk. Fat yield is not greatly different because as milk yield declines, average fat test increases with advancing lactation (8), thus making the errors compensating to a certain extent.

The summary of analysis of variance using maximum deviations from actual yields for each of the 115 calculations on a total of 19 cows as shown in table 4 reveals a highly significant difference between cows for milk, fat, percentage fat and F.C.M. Percentage fat was more variable for twice daily milking ($P < 0.01$), which is in agreement with more detailed calculations presented earlier (8). The A.R. test was more accurate ($P < 0.01$) for estimating fat and F.C.M. yield, and the 48-hr. test was more accurate for estimating fat yield

TABLE 3

Comparison of period of month of test to average deviations[a] and total deviations from actual yield by four methods of calculating production records

Time of month	Type of test	Hours of test	Average[a] deviations				Milk deviations			Fat deviations			Butterfat test deviations			F.C.M. deviations		
							Maximum			Maximum			Maximum			Maximum		
			Milk	Fat	Test	F.C.M.	+	−	$\bar{x}$	+	−	$\bar{x}$	+	−	$\bar{x}$	+	−	$\bar{x}$
			(*lb.*)	(*lb.*)	(%)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(%)	(%)	(%)	(*lb.*)	(*lb.*)	(*lb.*)
A[b]	AR	24		−2.8	−0.023	− 43				19.4	25.3	10.6	0.168	0.191	0.074	291	380	159
		48		−4.2	−0.026	− 62				19.1	27.6	8.7	0.179	0.113	0.058	286	414	130
	HIR	24	+139	−0.7	−0.035	+ 54	574	1,085	297	27.9	38.5	10.3	0.090	0.182	0.067	557	1,012	240
		48	+124	−1.9	−0.038	+ 21	724	1,113	269	18.3	35.8	8.2	0.124	0.134	0.058	494	982	192
	Av.		+132	−2.4	−0.030	− 8			283			9.4			0.064			180
B	AR	24		−0.4	−0.003	− 6				32.5	27.5	9.5	0.200	0.187	0.063	488	396	143
		48		+2.0	+0.006	+ 30				30.6	18.9	11.3	0.232	0.186	0.078	459	306	170
	HIR	24	+121	+5.0	−0.002	+124	883	517	287	39.6	34.4	12.7	0.187	0.207	0.059	713	557	304
		48	+193	+8.8	+0.011	+209	885	428	298	31.9	26.8	15.0	0.203	0.131	0.077	771	573	322
	Av.		+157	+3.8	+0.003	+ 89			292			12.1			0.069			235
C	AR	24		+0.5	+0.002	+ 7				22.6	31.7	10.6	0.123	0.236	0.069	339	476	159
		48		−1.3	−0.008	− 19				11.6	29.9	7.0	0.088	0.223	0.047	174	448	105
	HIR	24	+234	+6.6	−0.011	+192	720	142	264	50.0	28.2	12.8	0.126	0.243	0.064	1,038	373	273
		48	+174	+1.0	−0.021	+ 96	535	191	214	19.5	23.6	8.4	0.087	0.217	0.042	477	304	196
	Av.		+204	+1.7	−0.010	+ 69			239			9.7			0.055			183
D	AR	24		−4.9	−0.039	− 74				25.6	26.4	9.6	0.072	0.202	0.064	384	396	144
		48		−5.8	−0.003	− 88				12.7	22.7	9.0	0.097	0.170	0.062	190	340	135
	HIR	24	− 60	−8.3	−0.047	−148	503	1,148	241	26.4	55.0	15.5	0.067	0.221	0.070	597	1,284	297
		48	− 23	−9.3	−0.047	−148	479	995	206	12.6	44.0	6.6	0.062	0.199	0.066	381	1,058	233
	Av.		− 42	−7.1	−0.034	−114			224			10.2			0.066			202
Av.	AR	24		−2.0	−0.017	− 31				32.5	31.7	10.2	0.200	0.236	0.068	488	476	153
		48		−2.6	−0.011	− 39				30.6	29.9	8.9	0.232	0.223	0.061	459	448	134
	HIR	24	+113	+0.4	−0.026	+ 55	383	1,148	272	50.0	55.0	12.4	0.187	0.243	0.065	1,038	1,284	273
		48	+118	−0.6	−0.024	+ 41	885	1,113	251	31.9	44.0	10.6	0.203	0.217	0.060	771	1,058	233
	Av.		+116	−1.2	−0.020	+ 6			262			10.5			0.064			198

[a] Plus and minus signs considered.

[b] 43 calculations, other times have 24.

A Testing dates selected at random throughout the month.
B Testing dates selected at random from the first 15 d. of each month.
C Testing dates selected at random from eighth to 22nd d. of each month.
D Testing dates selected at random from the last 15 d. of each month.

($P < 0.01$) and F.C.M. yield ($P < 0.05$) but did not show a significant difference for milk yield. The period of the month tested showed no significant difference for milk and fat test but was significantly different for estimating yield of fat and F.C.M.

SUMMARY

Individual milkings of 19 Holstein cows were weighed and tested for butterfat content throughout a 305-day lactation period. These data were used to compare the relative accuracy of the following methods of testing: (a) calendar month; (b) centering date for 30-, 60-, 90-, 120- and 150-day testing intervals with a 24- to 96-hr. continuous test at each testing interval; and (c) H.I.R. and A.R. using 24- and 48-hr. testing each month and testing dates selected at random from the first, middle and last half of the month and from the entire month.

The calendar month method showed twice as much variation as the centering date method, but the former is not likely to be in error more than ± 5 per cent for F.C.M. in 25 per cent of the records, nor more than ± 12 per cent in 1 per cent of the records. Herd averages of nine or more cows would not be in error more than ± 5 per cent 1 per cent of the time. The centering date on the same basis would show less than half this amount of variation.

The percentage error in estimating milk yield exceeded by 25 per cent of the records was 2.4, 3.4, 5.6, 7.4 and 8.8 for the 30-, 60-, 90-, 120- and 150-day testing intervals, respectively. Comparable error for fat yield was 1.2 to 2.5 per cent greater.

The effects of 24- to 96-hr. testing at each test interval reduced the error less than 1 per cent for milk and less than 2 per cent for fat when the 96-hr. test was compared with the 24-hr. test for each of the testing intervals studied.

The accuracy of the 90-, 120- and 150-day intervals of testing is grossly influenced by the stage of lactation in which the cow is first tested.

The A.R. method of testing (one test per month), which includes a preliminary milking and reporting of daily milk weights, is on the average only slightly

TABLE 4

Statistical summary of table 3

Variable	D.F.	Milk	Fat	Per cent fat	F.C.M.
Cows	18	**	**	**	**
Times milked	1	n.s.	n.s.	*	n.s.
A.R. × H.I.R.	1	.....	**	n.s.	**
24- × 48-hr. test	1	n.s.	**	n.s.	*
Period of month	3	n.s.	*	n.s.	*
Times milked × 24- and 48-hr. test	1	n.s.	n.s.	n.s.	n.s.
Times milked × A.R. and H.I.R.	1	.....	n.s.	n.s.	n.s.
A.R. and H.I.R. × 24- and 48-hr. test	1	.....	n.s.	n.s.	n.s.
Cows × A.R. and H.I.R.	18	.....	n.s.	n.s.	n.s.

** Significant at the 1% level of probability.
* Significant at the 5% level of probability.
n.s. Non-significant.
..... Comparison not applicable.

more accurate for estimating fat yield than the H.I.R. method and is less than 1 per cent more accurate in estimating F.C.M. yields.

Cows gain advantage when always tested at the first of the month compared with cows always tested at the end of the month. This is a source of variation when H.I.R. credits and D.H.I.A. credits are based on the same daily test period.

The data used in this study show good agreement with other published reports where comparisons can be made. This indicates the comparisons reported in this study would be applicable to average conditions.

REFERENCES

(1) Alexander, M. H., and Yapp, W. W. Comparison of Methods of Estimating Milk and Fat Production in Dairy Cows. J. Dairy Sci., 32: 621–629. 1949.

(2) Bayley, N. D., Liss, R. M., and Stallard, J. E. A Comparison of Bimonthly and Quarterly Testing with Monthly Testing for Estimating Dairy Cattle Production. J. Dairy Sci., 35: 350–355. 1952.

(3) Cannon, C. Y., Frye, J. B., and Sims, R. Predicting 305-day Yields from Short-time Records. J. Dairy Sci., 25: 991–999. 1942.

(4) Copeland, L. Monthly and Bi-monthly Tests. Jersey Bull, 47: 723. 1928.

(5) Dick, I. D. Some Results on the Accuracy of Milk Sampling over a Complete Lactation and the Daily Variability in Milk Yields. N. Z. Sci. Technol., 32(A): 25–29. 1950.

(6) Erb, R. E., Goodwin, Mary M., Morrison, R. A., and Shaw, A. O. Lactation Studies. I. Effect of Gestation. J. Dairy Sci., 35: 224–233. 1952.

(7) Erb, R. E., Goodwin, Mary M., Morrison, R. A., and Shaw, A. O. Lactation Studies. II. Effect of Estrus. J. Dairy Sci., 35: 234–244. 1952.

(8) Erb, R. E., Goodwin, Mary M., Morrison, R. A., and Shaw, A. O. Lactation Studies. III. Variations in Daily Yields of Dairy Cows. J. Dairy Sci., 35: 967–976. 1952.

(9) Gaines, W. L. Deferred Short-time Test as a Measure of the Performance of Dairy Cows. J. Agr. Research, 35: 237–247. 1927.

(10) Gifford, W. The Reliability of Bi-monthly Tests. J. Dairy Sci., 13: 81–90. 1929.

(11) Houston, J. The Errors Involved in Certain Methods of Estimating the Lactation Yield of Milk and Butterfat. J. Dairy Research, 4: 37–47. 1932.

(12) M'Candlish, A. C., and M'Vicar, A. Are Milk Record Association Results Accurate? Scot. J. Agr., 8: 201–205. 1925.

(13) McDowell, J. C. Testing Cows for Production Every Other Month. Holstein-Friesian World, 24: 1970. 1927.

(14) McKellip, I., and Seath, D. W. A Comparison of the Different Methods of Calculating Yearly Milk and Butterfat Records. J. Dairy Sci., 24: 181–192. 1941.

(15) Morrison, R. A. Centering Date Versus Calendar Month for Computing Dairy Cow Production Records. Master's Thesis, The State College of Washington, Pullman. 1949.

(16) Snedecor, G. W. *Statistical Methods Applied to Experiments in Agriculture and Biology.* 4th Ed. The Iowa State College Press, Inc., Ames. 1946.

(17) Yapp, W. W. Relative Reliability of Official Tests for Dairy Cows. Ill. Agr. Expt. Sta. Bull. 215. 1915.

THE EFFECT OF HEAT ON MILK AS A CULTURE MEDIUM FOR LACTIC ACID BACTERIA[1]

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Several decades ago it was assumed that bacteria grew better in raw than in heated milk, the growth becoming progressively poorer as the heat treatment was increased (2, 7). More recently the pendulum has swung in the opposite direction because practically all investigators of this subject have shown improved growth of organisms in milk subjected to heat treatments intermediate in severity beween ordinary pasteurization and momentary boiling. This improvement is attributed to the destruction of one or more inhibitory components naturally present in raw milk. Morris (9) lists three of these bactericidal or bacteriostatic factors, the most resistant being destroyed by heating to 70 to 75° C. for 30 to 60 min. (6, 10, 12).

The effect of temperatures above 70° C. has not been studied extensively but the available reports show considerable lack of agreement. For example, Hammer and Baker (5) and Rice (11) found little difference in acid production by lactic cocci in milk samples heated in the range 70 to 100° C. for various times. Knudsen and Sörensen (7), Bogdanoff (2), Orla-Jensen and Jacobsen (10) and Davis (3) present results that show steady improvement in acid production by certain lactic organisms as the heat treatment given the milk is increased even up to boiling for 2.5 hr. (3) or autoclaving for 15 min. at 115 to 120° C. (2, 7, 10). Singh and Laxminarayana (15) obtained much better growth of a lactic streptococcus starter in milk pasteurized at 145° F. for 30 min. than in raw milk, and momentary boiling caused a further improvement, especially during the first few hours of incubation. At the other extreme, Golding *et al.* (4) found the rate of acid production by a lactic starter culture to be much slower in milk heated to 100° C. or above for 30 min. than in milk receiving a milder treatment.

The study reported here was planned to investigate further the effect of relatively severe heating of milk on growth of some of the important species of lactic acid bacteria. The mildest treatment tried, 80° C. for 10 min., was selected on the basis that it should inactivate any known inhibitory substance occurring naturally in the milk. It had the further advantage of destroying vegetative contaminants which might grow and affect the test results. The other treatment used routinely, autoclaving at 115° C. for 15 min., was selected because it is a typical exposure to which milk is subjected during laboratory sterilization. After it was shown that most of the lactic organisms grew better in autoclaved milk, efforts were made to determine why the more severe heating was beneficial.

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EXPERIMENTAL METHODS

Milk from individual cows was obtained at the barn immediately after milking. Mixed milk was secured from the weigh tank or a holding tank at the University of Wisconsin dairy. The samples were allowed to cream overnight at 5° C., after which the fat was removed and the skimmilk was dispensed in sterile bottles or large test tubes. These were heated in a deep water bath or in an autoclave for the required time, cooled rapidly to the desired incubation temperature and inoculated with 1 per cent of a 24-hr. culture of the test organism. The cultures were incubated at 30 or 37° C. depending on their optima.

Samples of the cultures were removed aseptically at intervals during incubation and titrated with NaOH to the phenolphthalein end point. The results,

TABLE 1

Acid production by two strains of S. lactis in milk from individual cows

Milk from cow[a]	Per cent developed acidity[b]											
	S. lactis Iowa						*S. lactis* A-1					
	At 21 hr.			At 45 hr.			At 21 hr.			At 45 hr.		
	A[c]	80[d]	A-80[e]	A	80	A-80	A	80	A-80	A	80	A-80
7J	0.58	0.44	0.14	0.69	0.55	0.14	0.42	0.40	0.02	0.63	0.58	0.05
14J	0.43	0.14	0.29	0.51	0.31	0.20	0.30	0.15	0.15	0.45	0.27	0.18
22J	0.50	0.33	0.17	0.67	0.55	0.12	0.39	0.33	0.06	0.55	0.50	0.05
27J	0.56	0.32	0.24	0.62	0.50	0.12	0.41	0.33	0.08	0.58	0.49	0.09
32J	0.35	0.07	0.28	0.60	0.28	0.32	0.28	0.04	0.24	0.52	0.29	0.23
13A	0.56	0.24	0.32	0.68	0.44	0.24	0.33	0.24	0.09	0.58	0.42	0.16
36G	0.55	0.40	0.15	0.70	0.57	0.13	0.33	0.20	0.13	0.57	0.46	0.11
40G	0.37	0.07	0.30	0.55	0.17	0.38	0.27	0.07	0.20	0.50	0.25	0.25
57G	0.55	0.15	0.40	0.60	0.30	0.30	0.33	0.13	0.20	0.56	0.26	0.30
Av.			0.25			0.22			0.13			0.16

[a] J = Jersey, A = Ayrshire, G = Guernsey.
[b] Titratable acidity of the culture minus that of the milk at the time of inoculation.
[c] Milk autoclaved for 15 min. at 115° C.
[d] Milk heated at 80° C. for 10 min.
[e] Value for autoclaved milk minus that for 80° C. milk.

calculated as lactic acid, are expressed as per cent developed acidity to eliminate the effect of differences in initial acidity of the milk caused by variations in the heat treatments. This figure was obtained by subtracting the initial titratable acidity of the heated milk from that of the culture.

RESULTS

Acid production by various lactic acid bacteria in heated milk. Table 1 shows that two strains of *S. lactis* produced more acid in autoclaved milk from individual cows than in the milk heated to 80° C. The differences between the values for the two heat treatments, as shown in the columns headed A-80, were greater for the more active Iowa strain than for *S. lactis* A-1. This effect of strong heating was not limited to milk from individual cows but also was shown with herd milk and with the mixed milk supply of the dairy (table 2).

TABLE 2

Acid production by S. lactis Iowa in mixed milks

Source of milk[a]	Trial no.	Per cent developed acidity[b]					
		At 21 hr.			At 45 hr.		
		A[c]	80[d]	A-80[e]	A	80	A-80
Herd no. 1	1	0.33	0.19	0.14	0.52	0.38	0.14
Herd no. 2	1	0.26	0.15	0.11	0.48	0.32	0.16
Herd no. 3	1	0.30	0.15	0.15			
Herd no. 4	1	0.29	0.17	0.12	0.52	0.36	0.16
	2	0.47	0.16	0.31	0.60	0.30	0.30
U.W. dairy mixed milk supply	1	0.30	0.19	0.11	0.54	0.34	0.20
	2	0.53	0.20	0.33	0.68	0.34	0.34
	3	0.41	0.20	0.21	0.61	0.51	0.10
	4	0.50	0.18	0.32	0.67	0.33	0.34
	5	0.49	0.35	0.14	0.66	0.56	0.10

[a] Samples of herd milk were taken from the weigh tank. The mixed milk was sampled from the holding tank.

[b–e] See footnotes to table 1.

Results with other strains of *S. lactis* and other species of lactic organisms are shown in table 3. These figures are the average values of two or three experiments with each organism, but individual trials agreed closely. All of the cultures except *S. liquefaciens* and *Lactobacillus casei* grew much better in the autoclaved milk than in that heated to 80° C. *S. lactis* 11 grew slowly and therefore showed a low A-80 value at 21 hr., but the usual difference was apparent at the later reading.

Inspection of the A-80 values in tables 1, 2 and 3 shows that the magnitude of the differences between the heat treatments at the 21-hr. reading remained

TABLE 3

Acid production by different lactic acid bacteria in mixed milk

Organism	Per cent developed acidity[a]					
	At 21 hr.			At 45 hr.		
	A[b]	80[c]	A-80[d]	A	80	A-80
S. lactis Iowa	0.47	0.16	0.31	0.61	0.30	0.31
" " A-1	0.35	0.12	0.23	0.48	0.24	0.24
" " 11	0.07	0.03	0.04	0.39	0.12	0.27
" " BCD	0.40	0.11	0.29	0.51	0.22	0.29
" " Rogers	0.25	0.11	0.14	0.42	0.23	0.19
" " Hammer	0.25	0.05	0.20	0.88	0.50	0.38
" " 30-5	0.35	0.16	0.19	0.52	0.31	0.21
" " 16	0.29	0.09	0.20	0.44	0.20	0.24
S. thermophilus	0.54	0.22	0.32	0.64	0.40	0.24
S. durans	0.42	0.24	0.18	0.50	0.45	0.05
S. liquefaciens S	0.47	0.70	*−0.23*	0.61	0.75	*−0.14*
" " 815	0.48	0.71	*−0.23*	0.62	0.73	*−0.11*
L. casei	0.18	0.23	*−0.05*	0.80	0.91	*−0.11*
L. lactis	0.69	0.12	0.57	1.04	0.44	0.60
L. acidophilus	0.73	0.12	0.61	1.15	0.33	0.82
L. bulgaricus	1.56	1.06	0.50	2.38	1.51	0.87

[a–d] See footnotes b to e, respectively, in table 1.

reasonably constant through 45 hr. In a few experiments the incubation period was extended to 3 or 4 days without changing the A-80 values appreciably. Thus, it is apparent that autoclaving caused more than a mere transitory effect. It produced a change of some kind that permitted a greater total amount of acid to be formed in the milk.

Effect of time and temperature of heating milk on acid production by S. lactis. Figure 1 shows the effect of heating mixed milk to 80, 100 and 115° C. for different time periods. Acid production by the test organism, *S. lactis* Iowa, increased steadily up to a certain point as the time of heating was lengthened. This beneficial effect accelerated as the temperature was increased.

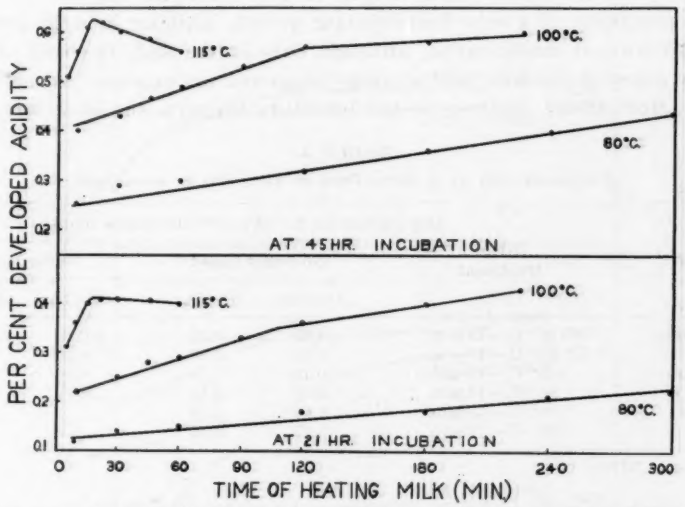

FIG. 1. Acid production by *S. lactis* Iowa in skimmilk heated at different temperatures for different times. (Results of a typical experiment.)

Excessive heating, however, was deleterious. As can be seen from the curve for 115° C., autoclaving for more than 15 to 20 min. resulted in decreased acid formation and boiling for more than 100 to 120 min. caused a decrease in the rate of improvement. Orla-Jensen and Jacobsen (10) also observed reduced acid formation in grossly overheated milk.

Why strong heating improves milk as a culture medium. Few of the workers who have demonstrated improved growth of bacteria in milk heated to temperatures higher than are necessary to inactivate the known thermolabile growth inhibitors have made a serious effort to determine specifically why the increased heating is beneficial. Most of the explanations are offered in the form of conjectures based on circumstantial evidence rather than on definitive experimental trials. Suggested reasons for the beneficial effect of strong heat treatments involve one or more of the following: (a) Reduction of the oxidation-reduction potential (15); (b) inactivation of a very heat-resistant inhibitor (10); or (c)

changing certain of the milk constituents to a form more readily usable by the organisms. Constituents that have been mentioned are lactose (10, 15), albumin (2) and proteins (10, 14, 15).

Since strong heating drives the oxygen out of milk and causes an increase in the number of free -SH groups, the resulting reduced environment might be expected to favor growth of the microaerophilic lactic acid bacteria. Orla-Jensen and Jacobsen (10), however, obtained no differences in acid production by lactobacilli in heated milk samples incubated with varying amounts of surface exposed to the air. In the study reported here there was no appreciable difference in the amounts of acid formed in autoclaved milk exposed to air for periods ranging from a few minutes to as long as 12 days before inoculation.

The possibility of a very heat-resistant growth inhibitor in milk cannot be discarded without consideration, although Orla-Jensen and Jacobsen (10) are the only investigators who have seriously suggested the existence of such a substance. Most of the evidence in the literature supports the idea that strong

TABLE 4

Acid production by S. lactis Iowa in whey and in mixed milk

Medium	Heat treatment	Per cent developed acidity			
		No buffer added		Buffer added[a]	
		At 21 hr.	At 45 hr.	At 21 hr.	At 45 hr.
Rennet whey	115° C.—15 min.	0.21	0.23	0.22	0.34
	80° C.—10 min.	0.16	0.19	0.18	0.38
Acid whey	115° C.—15 min.	0.10	0.14	0.10	0.26
	80° C.—10 min.	0.12	0.18	0.11	0.27
Skimmilk	115° C.—15 min.	0.50	0.65		
	80° C.—10 min.	0.17	0.42		

[a] 0.5% K_2HPO_4.

heating improves milk as a culture medium by making available food materials that the organisms cannot obtain from milk as secreted. There is no agreement, however, as to what these food materials might be.

As a preliminary step to see which part of milk is influenced favorably by strong heating, several strains of *S. lactis* were grown in whole milk and in some of the same milk with the fat removed. Portions of each were heated to 115° C. for 15 min. and to 80° C. for 10 min. As usual, the organisms grew much better in the autoclaved milk, but the results for whole and skimmilk were identical. Therefore it was concluded that the beneficial effect of heat had no relation to the fat.

In the next experiments the casein was removed from skimmilk by isoelectric precipitation with lactic acid and by coagulation with rennet. The resulting wheys were adjusted to pH 6.5 before and again after the customary heat treatments. A little more acid was formed in the rennet than in the acid whey (table 4), but there was no marked difference in the results for the two heat treatments. In early experiments the total amount of acid produced in the two wheys was low because the pH soon reached a limiting value (4.4 to 4.6) owing to the low buffering action. When 0.5 per cent K_2HPO_4 was added (table 4)

the total amount of acid formed was increased, but still there was no significant difference between the heat treatments. Autoclaved skimmilk, on the other hand, allowed the usual better growth. Thus, it is apparent that the beneficial effect of strong heating is lost when the casein is removed. The results with whey seem to show definitely that the albumin, globulin and lactose are not involved appreciably in this action of heat.

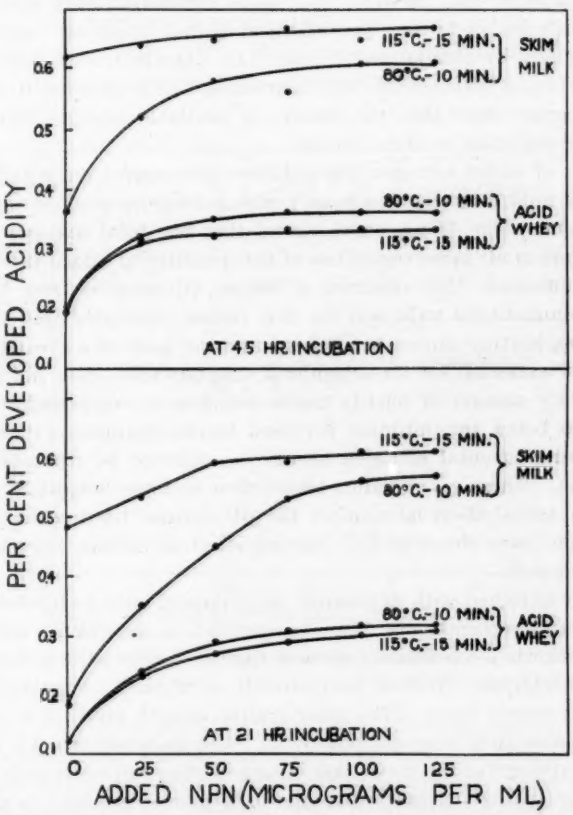

FIG. 2. Acid production by *S. lactis* Iowa in heated skimmilk and in whey enriched with different amounts of supplemental nitrogen in the form of Difco peptonized milk. (Results of a typical experiment.)

If it is assumed that strong heating is beneficial because it provides an increased amount of readily usable nitrogen sources for the organisms by partial hydrolysis of the casein, then the addition of available nitrogen compounds to milk heated at 80° C. for 10 min. should make it as good for *S. lactis* as autoclaved milk. If, on the other hand, autoclaving makes possible better growth by inactivating a heat-resistant inhibitor (10), then the addition of supplementary nitrogen to milk heated to 80° C. should effect no improvement.

Figure 2 shows the results obtained when different amounts of a heat-steri-

lized solution of Difco peptonized milk were added aseptically to milk and whey samples that had been heated as usual. Similar results were obtained by adding peptic digest of vitamin-free casein. Small amounts of supplemental nitrogen caused a tremendous increase in acid production in skimmilk heated to 80° C. Addition of only 60 to 65 γ per milliliter allowed as much acid to develop at 21 hr. as occurred in autoclaved milk with no addition. In this connection it should be mentioned that the NPN content of autoclaved milk exceeded that of the 80° C. milk by 55 to 57 γ per milliliter in two trials, as measured by the method described by Baribo and Foster (1). Quantities of peptonized milk greater than 75 γ of nitrogen per milliliter caused little increase in acidity, indicating that factors other than the amount of available nitrogen were regulating growth of the organism in these samples.

Up to 50 γ of added nitrogen per milliliter also caused an initial stimulation in autoclaved milk, but this was mostly just a temporary effect on the *rate* of acid production. The 45-hr. curve shows that the total amount of acid was nearly the same in all tubes regardless of the quantity of added nitrogen. Orla-Jensen and Jacobsen (10) observed a similar stimulation from adding yeast autolysate to autoclaved milk and for this reason concluded that the beneficial effect of strong heating cannot be explained on the basis of a greater quantity of available food material for the organisms. Apparently, they did not consider the fact that the amount of readily usable nitrogenous compounds even in autoclaved milk is below the optimum for most lactic organisms and therefore the addition of supplemental nitrogen should be expected to stimulate the initial rate of growth. These investigators tested their cultures only at 18 to 24 hr. If they had also tested them later, after the pH became limiting to growth, they probably would have observed little lasting effect of adding yeast autolysate to the autoclaved milk.

Acid whey enriched with peptonized milk showed only a slight difference between the heat treatments (fig. 2). In contrast to the results with skimmilk, autoclaved acid whey consistently showed slightly poorer acid development, suggesting the deleterious effect of heat already mentioned. Similar results were observed with rennet whey. The total acidity in both kinds of whey was relatively low because they were not buffered. Their final pH was 4.4 to 4.6.

Further support for the thesis that strong heating improves milk as a culture medium by providing increased amounts of available nitrogen is given by the results in fig. 3. As usual, the test organism produced relatively little acid in milk heated to 80° C. for 10 min. When autoclaved milk was added to the less strongly heated milk in greater and greater quantities, however, acid production improved steadily. This is believed to mean that the amount of added available nitrogen in the autoclaved milk determined the rate and amount of acid formed. With mixtures containing greater than 2:1 ratios of autoclaved milk to that heated at 80° C. for 10 min., no further improvement resulted, indicating that some factor other than the available nitrogen supply limited acid production in these mixtures. If the improvement caused by adding more and more autoclaved milk were a simple result of diluting a heat stable inhibitor in the less strongly

heated milk, acid production should have continued to increase to a maximum in the sample containing autoclaved milk alone.

DISCUSSION

This report confirms earlier findings to the effect that milk is not always a good culture medium for certain of the lactic acid bacteria, even when it is heated only enough to inactivate the inhibitory constituents normally present. The evidence fails to support the suggestion that milk contains an especially heat-resistant inhibitory material (10). It is believed instead that improved growth in strongly heated milk results from a greater supply of readily available nitrogenous compounds.

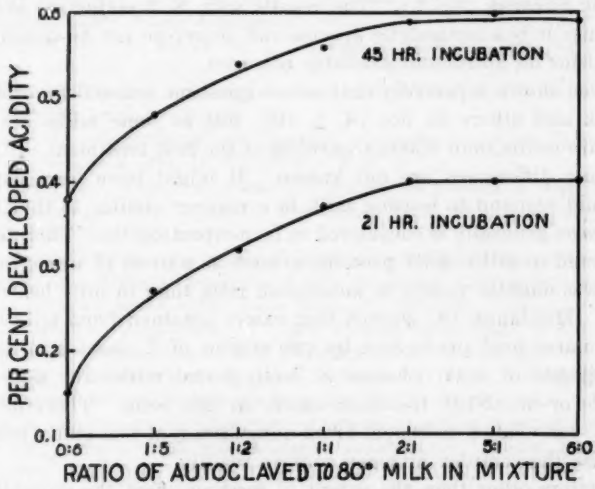

FIG. 3. Acid production by *S. lactis* in different mixtures of skimmilk heated to 80° C. for 10 min. and to 115° C. for 15 min. (Results are the averaged values for four trials.)

The source of these compounds is most likely the milk proteins. The results do not show conclusively whether one or more proteins is involved, but from the data one must believe that casein is primarily concerned. Since denaturation or cooking of some proteins increases their digestibility, autoclaving might be expected to make the albumin and globulin more readily usable by the bacteria. The results with whey do not show this, however. Studies on the availability of individual heated proteins to microorganisms might explain specifically the beneficial effect of strong heat on milk.

Even less is known about the nature of the deleterious effect of heat than about its beneficial action. Without citing evidence, Singh and Laxminarayana (15) suggest that some of the breakdown products of lactose and protein produced as a result of heating may become inhibitory to the growth of starter organisms. It might be just as logical to speculate that heat destroys some essen-

tial growth substance present in milk in limited quantity. This study has revealed nothing that would help to explain the basis of the deleterious action.

The beneficial effect of heat is noticeable at a lower temperature or in a shorter time than the deleterious effect (fig. 1). Both reactions probably proceed simultaneously but at different rates, and the former outweighs the latter at first because the amount of available nitrogen in the milk is the main factor regulating growth. As heating goes on the amount of available nitrogen continues to increase, but eventually the deleterious action reaches a limiting point so that it, rather than the amount of available nitrogen, controls the growth of the organisms. The data for *S. liquefaciens* (table 3) show the deleterious effect in progress even with a treatment of 15 min. at 115° C., which was near the optimum for *S. lactis* (fig. 1). The results with *S. liquefaciens* are not unexpected because it is a proteolytic species and therefore not as dependent as the other organisms on additional available nitrogen.

It has been shown repeatedly that some organisms respond favorably to autoclaving milk and others do not (4, 7, 10), just as some milks are inherently poorer culture media than others regardless of the heat treatment (10, 13). Reasons for these differences are not known. It might have been expected that *L. casei* would respond to heating milk in a manner similar to that of *S. lactis*, because *L. casei* generally is considered to be nonproteolytic. Therefore it would not be expected to utilize milk proteins as such as sources of nitrogen. Even so, its growth was slightly poorer in autoclaved milk than in milk heated to 80° C. for 10 min. Middaugh (8) showed that casein obtained from milk by centrifugation stimulated acid production by two strains of *L. casei* in a nitrogen-deficient ultrafiltrate of milk, whereas *S. lactis* tested under the same conditions showed little or no ability to utilize casein in this form. Therefore, it is possible that *L. casei* failed to benefit from autoclaving in this study because it was able to utilize the complex nitrogen sources readily.

Many factors other than the extent of heating affect the suitability of milk as a culture medium for bacteria. For example, growth of the natural contaminants prior to heating often effects changes which make the milk a better or poorer medium for starter organisms, regardless of the heat treatment applied. It has been observed frequently in this study and in others (7, 10) that milk which has been aged or otherwise held so that the normal contaminants can grow is a better medium than milk handled under ideal conditions. Only with the latter are the beneficial effects of strong heat observed consistently. As the bacteriological quality of milk continues to improve the importance of the heat treatment given milk for starter cultures doubtless will become increasingly important.

SUMMARY

Six of eight species of homofermentative lactic acid bacteria tested grew much better in milk heated at 115° C. for 15 min. than in milk heated to 80° C. for 10 min. The evidence indicates that milk is deficient in readily available nitrogen sources for these organisms and that the improved growth in autoclaved milk was the result of partial hydrolysis of the casein.

In addition to the beneficial effect of heating, a deleterious action was demonstrable if the milk was grossly overheated.

REFERENCES

(1) Baribo, L. E., and Foster, E. M. The Intracellular Proteinases of Certain Organisms from Cheese and their Relationship to the Proteinases of Cheese. J. Dairy Sci., **35**: 149–160. 1952.

(2) Bogdanoff, W. M. L'influence des différentes températures de pasteurization du lait sur le développement des microbes acido-lactiques du type *Streptococcus lactis* et *Bacterium casei*. Lait, **13**: 677–686. 1933.

(3) Davis, J. G. Present-day Problems in Cheesemaking. Dairy Inds., **5**: 212–217. 1940.

(4) Golding, N. S., McCorkle, L., and Milich, F. Some Factors which May Affect Activity Tests for Lactic Starters. Proc. 32nd Annual Meeting, Western Division, American Dairy Science Association. 1951.

(5) Hammer, B. W., and Baker, M. P. Studies on Starters. Iowa Agr. Expt. Sta. Research Bull. 106. 1928.

(6) Hobbs, B. C. The Part Played by Bacteria in the Reduction of Methylene Blue in Milk. J. Dairy Research, **10**: 35–58. 1939.

(7) Knudsen, S., and Sörensen, A. Beiträge zur Bakteriologie der Säurewecker. Zentr. Bakt. Parasitenk., Abt. II., **79**: 75–85. 1929.

(8) Middaugh, P. R. Utilization of the Nitrogenous Constituents of Milk by *Streptococcus lactis* and *Lactobacillus casei*. Unpublished Ph.D. thesis. Library, University of Wisconsin, Madison. 1951.

(9) Morris, C. S. The Presence in Raw Milk of a Bactericidal Substance Specific for Certain Strains of Coliform Organisms and the Comparative Rate of Growth of Bacteria in Raw and Pasteurized Milk. Dairy Inds., **10**: 180–181. 1945.

(10) Orla-Jensen, S., and Jacobsen, J. Neue Untersuchungen über die bakteriziden Eigenschaften der Milch. Zentr. Bakt. Parasitenk., Abt. II., **80**: 321–341. 1930.

(11) Rice, E. B. Factors Influencing Acid Production by Lactic Acid Streptococci in Milk and Cheese Curd. J. Australian Inst. Agr. Sci., **8**: 28–29. 1942.

(12) Ritter, P. Einfluss der Erwärmung von aseptisch ermolkener Rohmilch auf das Wachstum der wichtigsten Milchsäurebakterienarten und *Esch. coli*. Landw. Jahrb. Schweiz., **59**: 915–928. 1945.

(13) Ritter, P. The Influence of Feeding and Other Factors on the Development of Bacteria in Milk, Important for Cheese Production. Dairy Sci. Abs., **11**: 224. 1950.

(14) Rowland, S. J. The Heat Denaturation of Albumin and Globulin in Milk. II. Denaturation and Degradation of Protein at Temperatures of 75–120° C. J. Dairy Research, **8**: 1–5. 1937.

(15) Singh, K., and Laxminarayana, H. Studies on the Growth of Starter Organisms in Abnormal Milk. Indian J. Dairy Sci., **1**: 78–86. 1948.

A COMPARISON OF "HARD" AND COMMERCIALLY SOFTENED WATER IN THE RATION OF LACTATING DAIRY COWS

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The value of soft water consumed by lactating dairy cows has never been investigated. A few isolated observations have been reported which appear to show increases in milk production when the cows are permitted to consume soft water. A search of the literature fails to bring to light any data which substantiate or refute these claims.

Leitch and Thomson (4) reviewed data on the effect on animals of consuming water which has a high saline and mineral content. They reported that this water was detrimental to animals, and excesses of nitrates, sulphates and sulphides, fluorine and magnesium caused certain disorders and even death when consumed by some animals. The effect of alkali water on dairy cows was reported by Larsen and Bailey (3) in 1913. This water was high in minerals, especially sodium and sulphur. No adverse effects were observed when this water was consumed by dairy cows. In certain areas of the United States and other parts of the world, the high mineral content of the water consumed by animals is of potentially great economic importance.

EXPERIMENTAL PROCEDURE

In order to test the value of soft water compared to the so-called hard water, an experiment using lactating dairy animals was started late in 1951. Twelve lactating cows were divided into six pairs of two animals each. The cows in each pair were as near alike as possible in age, milk production, size and stage of lactation. One cow in each pair then was placed on the hard water treatment and the other cow on a commercially-softened-water treatment. After an adjustment period of 16 days, an experimental period of 57 days followed. At the end of this period, the treatments were reversed and a change-over period of 5 days was followed by an experimental period of 57 days.

All cows were weighed on the first and last 3 days of each experimental period. Daily milk production of each cow was recorded and 1-day composite milk samples were taken at 10-day intervals for the determination of butterfat content.

The cows received an allotment of 10 lb. of a top no. 2 alfalfa hay and 30 lb. of late-cut, well-eared corn silage. The concentrate mixture consisted of ground corn, ground oats, wheat bran, cottonseed meal, iodized salt and steamed feed bone meal. The analysis of the hay, silage and concentrate used in the experiment is shown in table 1. Amount of concentrate fed was based on the requirements for milk production and body maintenance at the start of the experiment and was reduced at the rate of 9 per cent at the end of each 30-day period.

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TABLE 1

The composition of the feeds used

Feed	Dry matter	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract
	(%)	(%)	(%)	(%)	(%)
Hay	86.62	17.69	2.79	23.18	49.77
Silage	26.33	8.81	3.28	21.11	61.55
Concentrate	87.54	19.63	4.64	7.19	63.69

A water meter was installed at the inlet of the supply of water for each of the groups. Individual water consumption was not determined.

Digestion and nitrogen balance trials were conducted for a 5-day period on one cow from each treatment, following the technique outlined by Eheart *et al.* (2).

The health of the animals was excellent throughout the experiment. The daily feed allotments were consumed completely by all the cows. One cow developed a slight lameness for several days. The cows were placed in a dry lot daily for about 2 hr. for exercise. Individual drinking cups were provided for the cows and they were allowed to consume water free choice.

The water supply was tested frequently throughout the experiment for hardness by the method of Betz and Noll (1). The soft water was produced by the zeolite process in which the Ca and Mg ions are replaced by Na ions. The water was from wells located on the college campus. The total hardness of the hard water averaged 290.4 ppm. nearly equally divided as to Ca and Mg ions. When this water was softened by the zeolite process, the total hardness was 0.0 ppm.

The summary of the data obtained is shown in table 2. No significant differences were observed in the changes in body weight or in the production of 4 per cent F.C.M. between the two treatments. The cows on the hard water

TABLE 2

The changes in body weight, feed and water consumption and amount of milk produced by lactating cows on the two treatments

	Treatment	
	"Hard" water	"Soft" water
Cows (*no.*)	12	12
Days (*no.*)	57	57
Body weight, initial (*lb.*)	1,087	1,087
Body weight, final (*lb.*)	1,095	1,088
Gain in body weight (*lb.*)	8	1
Feed consumption/day/cow		
Hay (*lb.*)	10	10
Silage (*lb.*)	30	30
Concentrate (*lb.*)	12	12
Water consumption/day/cow	97.1	97.1
Production of 4% F.C.M./day/cow	33.0	33.2
Ratio: $\frac{\text{water consumed}}{\text{milk produced}}$	2.94	2.92
Ratio: $\frac{\text{water consumed}}{\text{dry matter intake}}$	2.87	2.87

treatment gained an average of 8 lb. for the period and the cows on soft water gained 1 lb. The production of milk between the two treatments was very close, there being only 0.2 lb. difference in the average daily production, which was not significant. There was no difference in feed consumption, dry matter intake or average daily consumption of water.

The digestion coefficients obtained by placing one cow from each treatment on a digestion trial of 5 days were very similar. The coefficients of the dry matter, crude protein, ether extract, crude fiber and nitrogen-free extract were 65.9, 67.8, 77.4, 51.1 and 71.0, respectively, for the cow on hard water, and 64.5, 68.2, 76.3, 45.4 and 70.6, respectively, for the cow on the softened water. The greatest difference was in the crude fiber digestion coefficients in which the hard water treatment showed an increase of 5.7 percentage points over the one obtained from the cow on the softened water.

SUMMARY

There were no significant differences observed between the two treatments in the production of 4 per cent F.C.M. or in changes in body weight. The cows while on the hard water produced 33.0 lb. of milk daily and gained an average of 8 lb. in body weight each. The cows while on softened water produced 33.2 lb. of milk daily and gained an average of 1 lb. in body weight each during the experimental period. No difference was observed in the average daily water consumption. All the cows consumed an average of 97.1 lb. of water daily regardless of the total hardness of the water. The ratio of water consumed to milk produced was 2.9 and the ratio of water consumed to dry matter intake also was 2.9.

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REFERENCES

(1) Betz, J. D., and Noll, C. A. Total-hardness Determination by Direct Colorimetric Titration. J. Am. Water Works Assoc., **42**: 49. 1950.

(2) Eheart, J. F., Holdaway, C. W., and Pratt, A. D. Outline of a New Technique for Digestion Trial Procedure. J. Dairy Sci., **28**: 839. 1945.

(3) Larsen, C., and Bailey, D. E. Effect of Alkali Water on Dairy Cows. S. Dak. Agr. Expt. Sta. Bull. 147. 1913.

(4) Leitch, I., and Thomson, J. S. The Water Economy of Farm Animals. Nutrition Abs. and Revs., **14**: 197–223. 1944.

UNIDENTIFIED DIETARY FACTORS IN DAIRY CATTLE NUTRITION. III. THE NUTRITIVE VALUE OF IMMATURE ALFALFA AND TIMOTHY HAYS FOR MILK PRODUCTION[1]

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Most of the information regarding the change in the nutritive value of legume and grass hays associated with the advance in maturity is based on chemical analyses and digestion trials. Woodman *et al.* (22) reported that all of the concentrates in the ration were satisfactorily replaced by immature grass for milk production. Newlander (18) replaced 5 lb. of hay with 4 lb. of artificially dried young grass in a ration which contained corn silage and grain and found that milk production was in favor of the young grass, although the number of pounds of fat-corrected milk (F.C.M.) per 100 lb. of total digestible nutrients (T.D.N.) was not altered significantly. Camburn (3) replaced the grain in a hay-corn silage-grain ration with young grass and found that when body weight and milk production were considered, the T.D.N. in dried grass were equal to or slightly better than the T.D.N. in the grain. Camburn (4) also replaced corn silage with soaked, artificially dried, immature grass in the ration of 10 cows during a 12-wk. period and reported that milk production increased in eight of ten cows, but since there was an increased intake of T.D.N. during the grass-feeding periods, no significant difference was found in the nutrients required to produce a unit of 4 per cent F.C.M. Camburn and Jones (5) reported further that the T.D.N. in artificially dried, immature forages were at least equal to the T.D.N. in grain for milk production when corn silage was in the ration.

Five feeding trials were conducted with milking cows at the Arizona Agricultural Experiment Station (1) to compare alfalfa cut in the bud-stage with that cut in the one-third bloom stage, but no difference in milk production could be determined. Similar results were reported by Brouwer and Dijkstra (2).

In previous papers (11, 12, 13), data were presented which showed that hay harvested in the bloom stage was deficient in the unidentified factor(s) supplied by grain. This paper reports the results obtained when immature alfalfa and timothy hays were compared with mature hays from the standpoint of the unidentified lactation factor(s).

EXPERIMENTAL

Seventeen cows (13 Holstein, 3 Brown Swiss and 1 Jersey) were used in 25 trials to study the unidentified factors in three early-bud alfalfa hays and two young timothy hays, one cut in the early-boot and the other in the preboot stage.

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Detailed data are presented for 12 trials, but the balance of the data are available for inspection. Each trial represents data from one cow.

Before it is possible to determine the relative values of bloom versus prebloom hay, it is necessary to use cows depleted of stored unidentified factor(s). This depletion technique has been described recently (11). In this experiment, the basal or bloom hay was used to deplete the cows before all or a part of the hay was replaced by the prebloom hay.

The period just prior to the replacement of the basal hay with the immature hay was used as the basal period for calculating the change in the production of 4 per cent F.C.M. (8). The basal periods varied from 9 to 15 days, whereas the periods in which the experimental hays were fed varied from 12 to 33 days. Short periods are preferable since the results are expressed as an increase or decrease in F.C.M. above or below that obtained for the basal period. Three-day composite milk samples were taken for butterfat determinations. The cows were weighed at the same hour every third day.

Basal hays no. 6 to 10, inclusive, were either first-cutting alfalfa-brome or second-cutting alfalfa harvested at one-tenth to full bloom and field-cured. Experimental hay no. 1 was first-cutting early-bud alfalfa-clover, harvested with a crusher and cured in windrows. Hay no. 2 was first-cutting alfalfa harvested in the early-bud stage and cured on a fence during a rainy period. Hay no. 3 was second-cutting early-bud alfalfa harvested from the same field during the same year as hay no. 2 and was field-cured. Timothy hay no. 4 was harvested in the early-boot stage and cured on a fence, while timothy hay no. 5 was cut in the preboot stage and cured in a swath under adverse weather conditions.

The coefficients of apparent digestibility were determined from digestion trials of all of the hays, except no. 6. These coefficients were used to calculate the T.D.N. and starch equivalents. The coefficients suggested by Morrison (17) were used for no. 6. The following formula was used for the calculation of starch equivalents:

$$\text{Starch equivalent} = \text{T.D.N.} - (0.58 \times \text{crude fiber})$$

Calculated net-energy (C.N.E.) was determined by the formula reported by Moore (16). Ballast was determined by the method reported in a previous paper (12) and is expressed as the per cent of the undigested carbohydrates. The corn used in trial 1 was U. S. no. 2. The coefficients suggested by Morrison were used to calculate the T.D.N. for corn. Trial 12 was added to illustrate the value of corn for supplying the unidentified factor(s) needed to balance the roughage portion of the ration.

RESULTS

The chemical composition, coefficients of digestibility, T.D.N. and starch equivalents are presented in table 1. The length of the experimental periods, days in lactation, body weights, F.C.M., amounts of hay consumed, T.D.N. and starch equivalent intake, crude fiber as a per cent of the dry matter, ballast, calculated net-energy and F.C.M. per 100 lb. of T.D.N. are shown in table 2. In trial no. 1, the cow increased 2.2 lb. of F.C.M. per day when first-cutting early-bud alfalfa (no. 1) replaced second-cutting alfalfa hay (no. 6). The T.D.N. and

TABLE 1

The description, chemical composition, coefficients of digestibility, T.D.N. and starch equivalents (S.E.) of the experimental and basal hays

Feed no.	Description	D.M.	Ash	Protein	Ether ext.	Crude fiber	N.F.E.	T.D.N.	S.E.
		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
				Experimental hays					
1	Alfalfa-clover, 1st-cut., early-bud crushed, 1936 crop	87.2[a]	7.25	14.7	1.89	24.2	39.2	50.2	36.2
		60	.....	68	16	50	70		
2	Alfalfa, 1st-cut., early-bud, 1941 crop	88.1	6.30	16.5	1.67	29.9	33.7	48.0	30.8
		55	.....	67	1	50	65		
3	Alfalfa, 2nd-cut., early-bud, 1941 crop	88.9	4.06	14.1	1.29	31.7	37.8	55.2	36.8
		63	.....	70	1	57	72		
4	Timothy, cut in boot-stage, 1941 crop	88.7	6.14	10.9	2.99	31.9	36.8	55.8	37.3
		65	.....	61	27	78	61		
5	Timothy, cut in pre-boot stage, 1942 crop	90.0	5.26	11.8	1.81	28.2	42.9	63.3	46.9
		73	.....	73	52	77	72		
				Basal hays					
6	Alfalfa, 2nd-cut., bloom stage, 1936 crop	87.4	7.19	15.8	2.18	25.4	36.8	50.7	36.0
		60[b]	.....	74	33	43	72		
7	Alfalfa-brome, 1st-cut., bloom stage, 1941 crop	89.5	5.61	12.3	1.90	30.7	39.0	49.0	31.2
		55	.....	64	32	47	65		
8	Alfalfa, 2nd-cut., bloom stage, 1941 crop	89.0	6.75	15.9	2.59	28.1	35.7	51.6	35.3
		60	.....	74	32	45	71		
9	Alfalfa-brome, 1st-cut., full bloom 1942 crop	87.8	6.20	12.7	1.53	33.0	34.4	45.5	26.4
		55	.....	63	0	50	61		
10	Alfalfa, 2nd-cut., bloom stage, 1942 crop	86.3	5.86	15.0	1.82	29.1	34.5	50.4	33.5
		56	.....	67	37	60	62		
				Corn					
1	Corn, U. S. no. 2, yel.	87.1	2.97	9.2	3.49	2.2	69.3	79.8	79.8

[a] The first line for each feed represents the chemical composition and the second line represents the coefficients of digestibility. All coefficients were obtained experimentally, except that line marked with a footnote [b] which represent Morrison's (17) values.

starch equivalents remained about the same, while the crude fiber, ballast and calculated net-energy were slightly lower on the early cut hay. In trials 2 and 3, a part of the basal hay (no. 7) was replaced by first-cutting early-bud hay (no. 2). Milk production increased in both trials, although the amounts of T.D.N., starch equivalents and calculated net-energy were lower. There was no significant change in crude fiber and ballast intakes. The amount of F.C.M. per 100 lb. of T.D.N. increased appreciably in both trials after replacing a part of first-cutting alfalfa-brome with early-bud alfalfa hay. In trial 3, F.C.M. re-

TABLE 2

The effect of replacing basal hays with immature alfalfa or timothy hay in an all-hay ration on milk yield

Trial	Exptl. period	In milk	Body wt.	Hay intake		Intake/day			Crude fiber in D.M.	Ballast	Yield of F.C.M.		Hay used
				Basal	Exptl.	T.D.N.	S.E.	C.N.E.			/day	/100 lb. T.D.N.	
(*no.*)	(*d.*)	(*d.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*therms*)	(%)	(%)	(*lb.*)	(*lb.*)	(*no.*)
1	15	164	1233	45.0	.....	22.8	16.2	17.5	29	40	23.0	101	6
	15	179	1260	.....	45.0	22.6	16.3	17.1	28	38	25.2	112	1
	12	194	1273	.....	30.0[a]	22.2	18.0	18.6	22	30	29.0	131	1
2	9	236	1007	38.7	.....	19.0	12.1	13.8	34	43	8.9	47	7
	15	245	1020	25.0	13.5	18.7	12.0	13.4	34	43	11.9	64	2
3	9	45	798	31.1	.....	16.0	11.0	12.6	32	40	20.0	125	7
	30	54	808	19.0	11.1	15.1	10.1	11.4	32	41	22.1	146	2
	15	84	808	15.0	9.8	13.2	9.0	10.7	33	38	22.0	167	4
4	15	57	1141	40.0	.....	20.6	14.1	16.2	32	40	27.6	134	8
	15	72	1146	24.1	15.0	19.6	13.1	14.8	32	41	25.2	129	2
	15	87	1134	.....	40.0	19.2	12.3	13.5	34	42	29.7	155	2
5	12	132	763	24.1	.....	11.0	6.4	6.9	38	44	13.0	118	9
	18	144	768	.....	24.8	11.9	7.6	8.4	34	42	16.0	134	2
6	15	255	1263	33.6	.....	16.5	10.5	12.0	34	43	16.6	101	7
	21	270	1255	20.0	12.8	16.9	10.9	16.7	35	40	18.8	111	3
7	12	134	1189	37.7	.....	19.4	13.3	15.2	34	42	17.9	92	8
	15	146	1152	21.5	15.0	19.4	13.1	15.7	33	38	17.1	88	3
8	15	150	999	39.5	.....	19.4	12.3	14.1	34	43	18.5	95	7
	30	165	1027	.....	41.1	22.9	15.3	19.6	36	34	20.7	90	4
	12	195	1039	.....	38.1	21.0	14.0	17.8	36	35	22.6	107	3
9	15	294	1239	38.4	.....	19.8	13.6	15.5	34	42	11.9	60	8
	18	309	1264	.....	39.5	22.0	14.7	18.9	36	34	13.5	61	4
	18	327	1258	.....	38.5	24.4	18.1	22.9	31	26	12.7	52	5
10	15	76	910	35.0	.....	17.6	11.7	10.1	34	39	17.3	98	10
	27	91	866	.....	28.7	16.4	10.9	14.0	36	31	17.9	112	4
11	15	171	977	40.0	.....	18.2	10.6	11.5	38	44	16.9	93	9
	15	186	1000	.....	35.0	19.5	13.1	16.7	36	34	19.6	101	4
12	15	102	1135	36.9	.....	18.7	12.8	14.3	31	41	16.5	88	.....
	15	117	1126	22.9[b]	.....	18.4	11.9	10.0	35	44	20.9	114	.....

[a] 9 lb. of corn added.

[b] 6.5 lb. of corn and 8 lb. of peanut hulls added, hay ration recorded in (13).

mained about the same when boot-stage timothy hay (no. 4) replaced early-bud alfalfa hay (no. 2). In trial 4, milk production decreased when 15 lb. of early-bud alfalfa hay (no. 2) replaced 15.9 lb. of basal hay (no. 8), but when all the basal hay was replaced by early-bud hay (no. 2), F.C.M. increased 2.1 lb. above the basal period, although the T.D.N., starch equivalents and calculated net-energy decreased and crude fiber and ballast increased.

When 24.1 lb. of first-cutting alfalfa-brome, full-bloom hay (no. 9) was replaced by 24.8 lb. of early-bud alfalfa hay (no. 2) in trial 5, F.C.M. increased 3 lb. per day. The T.D.N., starch equivalents and calculated net-energy values were higher, crude fiber and ballast were lower and the amount of F.C.M. per 100 lb. of T.D.N. increased.

First-cutting alfalfa-brome hay (no. 7) was used as the basal hay in trial 6, where a part of the basal hay was replaced by second-cutting early-bud alfalfa (no. 3). The amount of F.C.M. increased 2.2 lb. per day and the T.D.N., starch equivalents and calculated net-energy intakes increased. In trial 7, when second-cutting early-bud alfalfa hay (no. 3) replaced a part of second-cutting alfalfa hay (no. 8), F.C.M. declined, although the calculated net-energy increased slightly.

In trial 8, basal hay (no. 7) was replaced by early-boot timothy hay (no. 4) on an appetite basis. This resulted in a marked increase in T.D.N., starch equivalents and calculated net-energy, which accounted for the increase in F.C.M. Less milk, however, was produced per 100 lb. of T.D.N. When hay no. 4 was replaced by second-cutting early-bud alfalfa hay (no. 3), the amount of F.C.M. increased 1.9 lb. on less T.D.N., starch equivalents and calculated net-energy.

The relative appetite of the cow for hays no. 8, 4 and 5 was studied in trial 9, which accounts for the greater intake of T.D.N., starch equivalents and net-energy when basal hay no. 8 was replaced by the prebloom hays. When preboot timothy replaced boot timothy, F.C.M. declined, although the amounts of T.D.N., starch equivalents and net-energy increased markedly.

In trial 10, the replacement of 35 lb. of basal hay no. 10 with 28.7 lb. of boot-stage timothy hay resulted in an increase of 0.6 lb. of F.C.M., although the T.D.N. and starch equivalents were markedly less than that received in the basal period. The increase in F.C.M. was markedly less than the increase in net-energy would indicate.

The effect of replacing immature hays with corn is shown in trial 1. An illustration showing the results of replacing 14 lb. of hay with 6.5 lb. of corn and 8 lb. of peanut hulls is shown in trial 12. The chemical composition, T.D.N. and starch equivalents of the feeds used in this trial were feeds no. 6, 9 and 13 in table 1 of a previous publication (13).

DISCUSSION

A complete or partial replacement of the basal hay used to deplete milking cows of the unidentified lactation factor(s) with an experimental hay make it possible to evaluate the approximate grain equivalent of the experimental hay. The comparison, however, is only relative and depends on the amount of the

unidentified factor present in the basal hay. The replacement of second-cutting alfalfa hay (bloom stage) with early-bud alfalfa hay (no. 1) resulted in a marked increase in milk production. Also, when first-cutting early-bud alfalfa hay (no. 2) replaced a part of basal hay no. 7, which was first-cutting alfalfa brome, the amount of F.C.M. increased against the tide of declining lactation. Similar results were obtained when this immature hay replaced all or part of basal hay no. 8, which was second-cutting alfalfa hay. Both of these basal hays were harvested in the bloom stage. It appears from the limited data that these two basal hays were approximately equal in the unidentified grain factor. Unfortunately a direct comparison of these two hays was not made.

When second-cutting early-bud alfalfa hay (no. 3), which was harvested from the same field during the same year as the first-cutting early-bud alfalfa hay (no. 2), replaced all or part of the basal hays, there was an increase in F.C.M. in one trial and a decline in four trials. Nevertheless, the second-cutting early-bud alfalfa did not appear to contain as much as the unidentified lactation factor as first-cutting early-bud alfalfa hay (no. 2).

A comparison of boot-stage timothy with preboot timothy (trial 9) indicated that the more mature timothy, cut in the boot-stage, was superior to preboot stage timothy for milk production. Early-bud alfalfa hays no. 1 and 2 appeared to contain greater milk-producing powers than the basal hays cut in the bloom stage and the early-bud second-cutting alfalfa hay (no. 3).

These results suggest that the amount of unidentified lactation factor(s) in hays does not necessarily depend upon the stage of maturity. These findings are in agreement with those of Graves *et al.* (9), who reported that four cows fed immature grass hay alone averaged 403 lb. of butterfat per year, whereas four cows fed silage alone, made from immature grass, averaged 388 lb. of butterfat. Two of the cows that made records on the immature grass hay also made records on alfalfa hay (in bloom) alone. The cows averaged 437 lb. when fed alfalfa hay alone and 404 lb. when fed immature grass hay alone. The mature alfalfa hay appeared to be superior to the immature grass hay for milk production. Dawson *et al.* (6), however, reported that the yearly milk production was greater for initial-bloom than for half-bloom or full-bloom alfalfa, yet when the cows were changed from first-cutting to second-cutting alfalfa in 1936, milk production increased regardless of the stage of maturity. No grain was fed. The efficient production of milk on Western hay alone (9) also suggests that the unidentified factor(s) does not necessarily follow the stage of maturity, since the hay used probably was cut during the bloom stage. Willard (21) observed that cows changed from poor alfalfa hay alone to good alfalfa hay increased markedly in milk. This increase may have been due to greater appetite for the good hay.

Knott and Hodgson (14) compared immature, artificially dried pasture herbage with alfalfa hay in a trial conducted with two groups of two cows each by the reversal method. No grain was fed. The grass hay was fed at the rate of 6.53 lb. per day per cow. U. S. grade no. 1 alfalfa hay was fed *ad libitum*. Milk production was greater when artificially dried pasture grass was fed, but the efficiency of production remained about the same due to a greater intake of T.D.N. during the grass-feeding periods.

Several experiments have been conducted to determine the value of young forage for milk production when considerable amounts of grain sources of the unidentified lactation factor(s) were present. These results are of interest from the standpoint of comparing the T.D.N. in young grass with the T.D.N. in grain in order to further check the modern concept of a balanced ration as recently shown by Huffman and Duncan (11, 12) and Huffman *et al.* (13). Knott and Hodgson (14) reported that when a grain mixture containing 20 per cent of immature, artificially dried pasture grass was compared with the basal ration there was no significant difference in milk production. Similar results were reported by Watson (20), Newlander *et al.* (18), Camburn (4), Camburn and Jones (5), Tinley and Bryant (19).

In this work, the partial or complete replacement of basal hays (depletion hays) by immature alfalfa and timothy hays did not always result in a decrease in the per cent of crude fiber in the dry matter. As a matter of fact, there was an increase in crude fiber in nine trials, a decrease in six and no change in ten trials. There appears to be no relation between the per cent of crude fiber in the ration and milk production. Milk production also failed to follow the calculated amounts of starch equivalents in the ration, which is in agreement with previous findings (12, 13). This is shown by increased production of F.C.M. in nine trials in which the starch equivalents decreased and decreases in F.C.M. in five trials when the starch equivalents increased. In 11 of 25 trials, milk production increased as the starch equivalents increased. The value of grain as a supplement to immature hay was included as an illustration of the efficiency of corn to supply the unidentified factor(s) needed to balance hay for milk production (trial 1). This factor(s) can not be explained adequately on the basis of T.D.N., starch equivalents, calculated net-energy, crude fiber or ballast, as was shown in previous reports (12, 13). These results lend further support to the modern concept of a balanced ration which was first expressed by Forbes (7) and officially defined by Mitchell (15) in 1935.

The results of this paper show that the unidentified lactation factor(s) content of herbage does not necessarily depend upon stage of maturity, crude fiber, ballast or T.D.N. content, on the dry basis. Further evidence was obtained also to show that the unidentified factor(s) in herbage can not be properly estimated by either the starch equivalent or calculated net-energy method of feed evaluation.

The lactation factor(s) in grain which balances the T.D.N. in hay appeared to be present in most of the immature hays when compared with mature basal hays. Whether to harvest hay in the immature stage or to use grain as a supplement to supply the unidentified lactation factor(s) is a question of agronomics and economics. These aspects were not studied in this investigation.

SUMMARY

Seventeen milking cows were used in 25 trials to determine the effect on milk production when all or part of a mature hay was replaced with an immature hay. In general, the immature hays stimulated milk production when they replaced part or all of the T.D.N. in mature hay and appeared to produce more milk per 100 lb. of T.D.N. Equally satisfactory or better results, however, can be ob-

tained on the same T.D.N. intake when less hay is fed, provided the ration is balanced with corn. Thus, no agronomic or economic implications concerning the use of early-cut forages are intended or implied.

Lack of exactness or fixed standards makes the arbitrary determination of the nutritive value of hay difficult. The apparent exceptional value, from the standpoint of coefficients of digestibility, starch equivalents and T.D.N., of the timothy hay cut in the preboot stage was not evident when fed as a supplement to a mature hay; however, if fed in other combinations, its value might become manifest. When corn replaced immature hay on an equal T.D.N. basis, there was a marked increase in milk production.

The results of this investigation provide further evidence that the amount of the unidentified lactation factor(s) in hay can not be estimated by either the T.D.N., starch equivalent or calculated net-energy system of feed evaluation.

REFERENCES

(1) Anonymous. Ariz. Agr. Expt. Sta., 45th Ann. Rpt., p. 49. 1934.

(2) Brouwer, E., and Dijkstra, N. D. Comparative Feeding Experiment to Test the Relative Values of Early- and Late-cut Hay for Dairy Cows. Verslag. Landb. Onderzoek., 45(C): 763–805. 1939. (Chem. Abs., 35: 1536. 1941.)

(3) Camburn, O. M. The Feeding Value of Artificially Dried Young Grass. II. Vt. Agr. Expt. Sta. Bull. 359. 1933.

(4) Camburn, O. M. The Feeding Value of Artificially Dried Young Grass. III. Vt. Agr. Expt. Sta. Bull. 368. 1934.

(5) Camburn, O. M., and Jones, C. H. Early Cut Artificially Dried Hays for Dairy Cows. Vt. Agr. Expt. Sta. Bull. 446. 1939.

(6) Dawson, J. R., Kopland, D. V., and Graves, R. R. Yield, Chemical Composition and Feeding Value for Milk Production of Alfalfa Hay Cut at Three Stages of Maturity. U.S.D.A. Tech. Bull. 739. 1940.

(7) Forbes, E. B. Net Energy of Completely Balanced Nutriment as a Measure of Nutritive Value of Rations and of Nutritive Requirement of Animals. Proc. Am. Soc. Animal Prod., 1932: 32–40. 1933.

(8) Gaines, W. L. The Energy Basis of Measuring Milk Yield in Dairy Cows. Ill. Agr. Expt. Sta. Bull. 308. 1928.

(9) Graves, R. R., Dawson, J. R., Kopland, D. V., Watt, A. L., and Van Horn, A. G. Feeding Dairy Cows on Alfalfa Hay Alone. U.S.D.A. Tech. Bull. 610. 1938.

(10) Graves, R. R., Dawson, J. R., and Kopland, D. V. Relative Values for Milk Production of Hay and Silage Made from Immature Pasture Herbage. U.S.D.A. Tech. Bull. 649. 1938.

(11) Huffman, C. F., and Duncan, C. W. The Nutritive Value of Alfalfa Hay. III. Corn as a Supplement to an All-alfalfa Hay Ration for Milk Production. J. Dairy Sci., 32: 465–474. 1949.

(12) Huffman, C. F., and Duncan, C. W. Unidentified Dietary Factors in Dairy Cattle Nutrition. 1. Digestibility of Peanut Hulls and their Use in "Ballast" Studies with Milking Cows Depleted on Hay Alone. J. Dairy Sci., 35: 30–40. 1952.

(13) Huffman, C. F., Duncan, C. W., and Chance, C. M. Unidentified Dietary Factors in Dairy Cattle Nutrition. II. Further Evidence of an Unidentified Factor(s) in Grain Needed to Balance Roughage for Milk Production. J. Dairy Sci., 35: 41–50. 1952.

(14) Knott, J. C., and Hodgson, R. E. The Feeding Value of Artificially Dried Pasture Herbage for Milk Production. J. Dairy Sci., 17: 409–416. 1934.

(15) Mitchell, H. H. Some Problems in the Study of Energy Metabolism. Report of the Conference on Energy Metabolism. The Committee on Animal Nutrition, National Research Council, 66–93. 1935.

(16) MOORE, L. A. A Relationship between Total Digestible Nutrients (T.D.N.) and Estimated Net Energy Values (E.N.E.) and a Possible Method of Estimating Net Energy Values with Producing Dairy Cows. U.S.D.A., Bur. Dairy Ind., BDI-Inf-114. Nov., 1950.

(17) MORRISON, F. B. *Feeds and Feeding.* 21st ed. The Morrison Publishing Co., Ithaca, New York. 1948.

(18) NEWLANDER, J. A. The Feeding Value of Artificially Dried Young Grass. Vt. Agr. Expt. Sta. Bull. 350. 1933.

(19) TINLEY, N. L., AND BRYANT, D. N. A Trial of the Feeding Value of Dried Grass and Sprouted Maize for Dairy Cows. J. Southeastern Agr. Coll., Wye, Kent, no. 42: 135–145. 1938.

(20) WATSON, S. J. Investigations into the Intensive System of Grassland Management. IV. The Digestibility and Feeding Value of Artificially Dried Grass. J. Agr. Sci., 21: 414–424. 1931.

(21) WILLARD, H. S. Roughage Feeding of Dairy Cattle. Wyo. Agr. Expt. Sta. Bull. 237. 1940.

(22) WOODMAN, H. E., BEE, J. W., AND GRIFFITH, G. Nutritive Value of Pasture. V. Pasture Grass Conservation: The Influence of Artificial Drying on the Digestibility of Pasture Herbage. J. Agr. Sci., 20: 53–62. 1930.

THE UTILIZATION OF UREA BY DAIRY CATTLE

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The value of urea in the ration of lactating dairy cattle has been reported by various workers (1, 7, 14, 15, 19). These reports show that the urea can be used effectively as a partial source of nitrogen and can replace at least partially the high protein supplements in the concentrate mixtures.

No data were found in the literature concerning the supplementation of the ration of dairy cows with DL-methionine when urea was included in the ration. However, Bailey and Ross (2) found that cattle apparently can use inorganic sulphates and that methionine had no apparent advantage over Na_2SO_4 when used in the ration. Other workers (10, 11) reported improved urea utilization when methionine was added to the ration of sheep. When an alfalfa hay ration of dairy cows was supplemented with 20 and 40 g. of L-cystine no benefit was observed (8). Similar results (18) were obtained with L-cystine when fed to sheep.

EXPERIMENTAL PROCEDURE

The data reported here covered three experiments conducted during the barn feeding period over a 3-yr. span. The experiments are designated as trials 1, 2 and 3 in the order in which they were completed. Twelve cows were used in each of the trials and divided into three groups of four cows each according to the experimental design outlined by Cochran *et al.* (4). Every cow was on each of the experimental rations used in a trial. An adjustment period of 10 days was followed by three 20-day experimental periods with a 5-day change-over period between the second and third experimental periods.

The cows were weighed at the beginning and end of each period on 3 consecutive days. Roughage consumption for each cow was held to a constant amount. Daily milk production weights were recorded on each cow and 1-day composite milk samples were taken at 10-day intervals to determine the butterfat content of the milk.

The levels of feeding were determined according to Morrison's (13) minimum standards for maintenance and milk production. The concentrate allowances were adjusted at the beginning of each experimental period on the basis of milk production and body weights during the adjustment and change-over periods, as recommended by Lucas (12).

Digestion trials were conducted during the last week of each experimental period according to the procedure outlined by Eheart *et al.* (5). During trial 1, three different cows were used for each digestion trial, while during trials 2 and 3, the same three cows were used for each digestion trial.

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The inclusion of some relatively high-fiber content roughage in all the rations was intentional. The compositions of the roughages, silages and concentrate mixtures used in the three trials are shown in table 1. The ingredients and the amounts used in each concentrate mixture are shown in table 2. The three concentrate mixtures used in each trial are identified as A, B and C. The concentrate mixture shown as A in each trial was considered the basal mixture.

All data were analysed statistically by the method of Snedecor (16).

TABLE 1

Composition of roughages and concentrate mixtures

Feed	Dry matter	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract
	(%)	(%)	(%)	(%)	(%)
		Trial 1			
Hay	94.27	18.25	1.95	27.81	44.55
Corn stover	89.79	4.63	1.58	38.48	51.79
Corn silage	24.84	7.19	2.97	23.60	61.38
Concentrate mixture A	94.21	17.13	4.67	11.08	63.14
Concentrate mixture B	93.48	18.81[b]	4.14	10.13	63.53
Concentrate mixture C[a]					
		Trial 2			
Hay	86.92	10.61	2.03	37.45	47.61
Corn silage	31.52	7.62	4.04	19.01	65.90
Concentrate mixture A	81.08	11.27	3.30	9.89	72.17
Concentrate mixture B	83.53	16.29	4.30	8.94	66.21
Concentrate mixture C	81.48	15.94[b]	3.34	8.87	63.38
		Trial 3			
Hay	84.64	10.19	1.44	37.28	44.91
Corn silage	22.80	8.61	2.66	28.27	56.59
Concentrate mixture A	87.92	16.13	4.42	5.90	69.32
Concentrate mixture B	87.74	14.63	3.94	10.66	66.90
Concentrate mixture C	87.69	20.69[b]	4.07	10.28	61.54

[a] Concentrate mixture C was concentrate mixture B to which 22 g. of methionine was added daily at feeding time.

[b] Includes urea nitrogen.

EXPERIMENTAL RESULTS

Trial 1. The data on body weight changes, feed consumption and milk production are summarized in table 3, trial 1. All the cows gained in weight during the experiment; however, there was no significant difference between the rations when these data were treated statistically. All cows consumed equal amounts of hay, corn stover and corn silage. The amounts of concentrate mixture consumed by the cows were approximately the same. Palatability was not a factor in the consumption of the concentrate mixture. The corn stover fed daily was limited to 4 lb., since the cows would refuse a considerable portion of the stover if more than 4 lb. per cow were fed daily.

The ration which contained the concentrate mixture B, in which urea replaced cottonseed meal, produced 530.5 lb. less 4 per cent F. C. M. and the ration containing concentrate mixture C, in which the urea was supplemented at the

rate of 22 g. of DL-methionine per cow per day, produced 148.4 lb. less 4 per cent F. C. M. than when the cows were on the basal ration. These differences were not significant.

The data obtained during the digestion trials are shown in table 4, trial 1. No significant differences were observed when these data were treated statistically.

TABLE 2

Formulas of the concentrate mixtures

Ingredients	Concentrate mixtures		
	A	B	C
	(*lb.*)	(*lb.*)	(*lb.*)
	Trial 1[a]		
Corn and cob meal	400	400	400
Ground oats	200	200	200
Cottonseed meal	150		
Urea		18	18
DL-Methionine			22[b]
Bone Meal	7.5	7.5	7.5
Salt	7.5	7.5	7.5
	Trial 2		
Corn and cob meal	400	400	400
Ground oats	200	200	200
Cottonseed meal		100	
Urea			12
Salt	6	7	7
Bone Meal	6	7	7
	Trial 3		
Corn and cob meal	500	500	500
Corncobs		200	200
Oats, ground	300	300	300
Cottonseed meal	180	180	180
Urea			20
Salt	10	10	10
Bone Meal	10	10	10

[a] Approximately 1,660,000 I. U. of carotene were added to each 100 lb. of concentrate mixture.

[b] 22 g. of DL-methionine were added to concentrate mixture B once daily.

Trial 2. The results of this trial are summarized in table 3, trial 2 and table 4, trial 2. There was an average loss in weight per cow on each of the rations. The greatest loss was observed on the basal ration which included the low protein concentrate mixtures. These differences were not significant.

The total production of 4 per cent F. C. M. was 5,831.1 lb. on the low-protein basal concentrate mixture. When this concentrate mixture was supplemented with cottonseed meal so that the crude protein was increased from 11.27 to 16.29 per cent, the production of milk was 6,332.0 lb. This was found to be a significant increase. Supplementing the basal concentrate mixture with urea to obtain a protein equivalent of 15.94 per cent also resulted in an increased production of milk. The total production of 4 per cent F. C. M. was 6,311.9 lb. on this ration. The increase in production over the basal ration also was significant.

There were no significant differences when milk production on the rations containing cottonseed meal or urea were compared.

There appeared to be a trend toward higher digestion coefficients of the feed constituents when the concentrate mixtures contained cottonseed meal or urea as shown in table 4, trial 2. The average coefficients were lowest on concentrate mixture A. However, when the data were treated statistically, no significant differences were shown between the rations.

TABLE 3

Daily feed consumption, liveweights, total and average daily milk yields

	Concentrate mixtures		
	A	B	C
	Trial 1		
No. of cows	4	4	4
No. of days	60	60	60
Initial wt. (*av. lb./cow*)	1,051.0	1,045.8	1,058.5
Final wt. (*av. lb./cow*)	1,068.2	1,072.8	1,066.5
Change in wt. (*av./cow*)	+17.2	+27.0	+8.0
Hay consumed (*lb./cow*)	8.0	8.0	8.0
Silage consumed (*lb./cow*)	30.0	30.0	30.0
Stover consumed (*lb./cow*)	4.0	4.0	4.0
Concentrates consumed (*lb./cow*)	11.4	11.1	11.3
Total 4% F.C.M. produced (*lb.*)	7,253.1	6,722.6	7,104.7
Daily 4% F.C.M. produced (*lb./cow*)	30.2	28.0	29.6
	Trial 2		
No. of cows	4	4	4
No. of days	60	60	60
Initial wt. (*av. lb./cow*)	1,089.9	1,078.1	1,078.8
Final wt. (*av. lb./cow*)	1,071.5	1,074.4	1,070.7
Change in wt. (*av. lb./cow*)	−18.4	−3.7	−8.1
Hay consumed (*lb./cow*)	12.0	12.0	12.0
Silage consumed (*lb./cow*)	30.0	30.0	30.0
Concentrates consumed (*lb./cow*)	8.3	8.3	8.3
Total 4% F.C.M. produced (*lb.*)	5,831.1	6,332.0	6,311.9
Daily 4% F.C.M. produced (*lb./cow*)	24.3	26.4	26.3
	Trial 3		
No. of cows	4	4	4
No. of days	60	60	60
Initial wt. (*av. lb./cow*)	1,192.4	1,192.0	1,192.8
Final wt. (*av. lb./cow*)	1,188.0	1,190.4	1,180.9
Change in wt. (*av. lb./cow*)	−4.4	−1.6	−11.9
Hay consumed (*lb./cow*)	9.9	9.2	9.8
Silage consumed (*lb./cow*)	30.0	30.0	30.0
Concentrates consumed (*lb./cow*)	17.2	17.0	16.4
Total 4% F.C.M. produced (*lb.*)	8,749.6	8,547.8	8,467.7
Daily 4% F.C.M. produced (*lb./cow*)	36.4	35.6	35.2

Trial 3. The changes in weights, feed consumption and milk production are shown in table 3, trial 3. There was an average loss in weight per cow on all the three rations studied. The greatest loss occurred on the ration containing the concentrate mixture C. The losses were 4.4, 1.6 and 11.9 lb. per cow. The differences in body weights between rations were not significant.

The total amount of 4 per cent F. C. M. produced was about the same for each ration. Excellent production was obtained on all the rations.

The average digestion coefficients of all the feed constituents (table 4, trial 3) except crude fiber, were lowest on the ration containing concentrate mixture B. The ration containing the highest protein concentrate mixture showed the highest digestion coefficient of all feed constituents except for nitrogen-free extract. When the data were treated statistically, no significant differences were apparent.

DISCUSSION

Trial 1. The results obtained in this trial on the use of urea as a source of nitrogen in the concentrate mixtures of dairy cattle are in line with the studies of Owen *et al.* (14), Rupel *et al.* (15), Archibald (1) and Willett *et al.* (19). Data with respect to milk production, body weights and digestibility coefficients

TABLE 4

The average coefficients of digestibility of the rations fed[a]

Feed constituents	Concentrate mixtures		
	A	B	C
	(%)	(%)	(%)
Trial 1			
Dry matter	60.52	61.88	62.86
Crude protein	59.28	62.77	63.37
Ether extract	73.15	74.71	74.20
Crude fiber	54.32	51.12	53.65
Nitrogen-free extract	64.99	66.57	67.06
Trial 2			
Dry matter	61.43	65.10	64.30
Crude protein	56.06	60.46	61.55
Ether extract	68.54	77.19	72.86
Crude fiber	45.39	51.47	51.59
Nitrogen-free extract	68.49	70.74	69.97
Trial 3			
Dry matter	61.87	60.45	62.49
Crude protein	58.18	57.59	63.83
Ether extract	77.49	74.90	77.83
Crude fiber	49.21	50.77	54.19
Nitrogen-free extract	67.88	65.48	65.61

[a] Based on the average of 3 cows for each ration.

show that urea can replace the nitrogen in cottonseed meal at the levels used in the present concentrate mixture. The cottonseed meal was estimated to provide approximately half of the crude protein in the concentrate mixture of the basal ration. In concentrate mixture B, urea was added to provide an equivalent amount of protein nitrogen. The urea was added at the rate of 3 per cent of the concentrate mixture. The addition of 22 g. of DL-methionine per cow per day to the urea-containing concentrate mixture did not result in any beneficial effects. These findings are not in agreement with the studies reported by Bailey and Ross (2) in which it was shown that cattle apparently can use inorganic sulphates and that methionine had no apparent advantage over Na_2SO_4. Huffman and Duncan (8) also found no apparent advantage when the ration of dairy cows was supplemented by L-cystine. Smuts and Marais (18) obtained similar results with sheep.

Trial 2. The results obtained from the ration containing the low-protein concentrate mixture are not particularly different from that expected. Work at this Station and elsewhere already has demonstrated that a sub-normal level of protein in the ration not only fails to meet the animal's protein needs, but also exerts a depressing effect on the digestibility of feed constituents. The object of the trial was to determine if urea was as effective as cottonseed meal in supplementing a low-protein concentrate mixture. In the concentrate mixture B, the cottonseed meal furnished approximately 30.8 per cent of the crude protein, and in concentrate mixture C, the urea furnished approximately 29.3 per cent of the protein equivalent. Briggs *et al.* (3), Johnson *et al.* (9), Harris and Mitchell (6) and Swift *et al.* (17) reported that the addition of urea to low-protein rations improved the utilization of these rations.

Trial 3. The results obtained in trial 2 led to the setting up of a trial that would determine the value of supplementing a ration adequate in protein to meet the animal's needs with urea to improve the utilization of the feed constituents. The second concentrate mixture was similar to the basal, except that 200 lb. of corncobs were added to each 1,000 lb. of mix, which lowered the crude protein content to 14.63 per cent and increased the fiber content to 10.66 per cent. Addition of 2 per cent urea to this ration constituted the concentrate mixture C., which contained 20.69 per cent crude protein and 10.28 per cent crude fiber. Based on protein equivalent, the urea furnished 29.3 per cent of the protein in this ration.

The inclusion of urea in the concentrate mixture did not result in increased milk production. The digestion coefficients of the constituents in the ration were not significantly different.

SUMMARY

Urea and cottonseed meal were of comparable value for milk production when incorporated into the concentrate mixtures at the levels used in this investigation. Addition of 22 g. of DL-methionine per cow per day to a ration containing urea did not produce any beneficial effects.

Addition of 200 lb. of ground corncobs per 1,000 lb. of concentrate mixture, so that the crude fiber content was doubled, did not adversely affect milk production or any of the other factors studied. Addition of urea to the corncob-concentrate mixture did not exhibit any beneficial effect on milk production or apparent digestibility of the feed constituents.

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REFERENCES

(1) ARCHIBALD, J. G. Feeding Urea to Dairy Cows. Mass. Agr. Expt. Sta. Bull. 406. 1943.
(2) BAILEY, S. H., AND ROSS, J. R. Ruminants Utilize Some Sulphur in Feedstuffs. Ore. Agr. Expt. Sta. Ann. Rpt. Bull., **461**: 1. 1948.

(3) Briggs, H. M., Gallup, W. D., Dinning, J. S., Darlow, A. E., Stephens, F. E., and Campbell, W. D. Feeding Trials with Sheep, Swine, and Beef Cattle. Okla. Agr. Expt. Sta. Misc. Pub. MP-13. 1948.

(4) Cochran, W. G., Autrey, K. M., and Cannon, C. Y. A Double Change-over Design for Dairy Cattle Feeding Experiments. J. Dairy Sci., **24**: 937. 1941.

(5) Eheart, J. F., Holdaway, C. W., and Pratt, A. D. Outline of a New Technique for Digestion Trial Procedure. J. Dairy Sci., **28**: 839. 1945.

(6) Harris, L. E., and Mitchell, H. H. The Value of Urea in the Synthesis of Protein in the Pouch of the Ruminant. I. In Maintenance. J. Nutrition, **22**: 167. 1941.

(7) Henke, L. A., Work, S. H., and Maruyama, C. Protein Supplements for Dairy Cattle. Hawaii Agr. Expt. Sta. Rpt. 1941–1942. 1943.

(8) Huffman, C. F., and Duncan, C. W. The Nutritive Value of Alfalfa Hay. I. Cystine as a Supplement to an All-alfalfa Hay Ration for Milk Production. J. Dairy Sci., **25**: 507. 1942.

(9) Johnson, B. C., Hamilton, T. S., Mitchell, H. H., and Robinson, W. B. The Relative Efficiency of Urea as a Protein Substitute in the Ration of Ruminants. J. Animal Sci., **1**: 236. 1942.

(10) Lofgreen, G. P., Loosli, J. K., and Maynard, L. A. The Influence of Protein Source upon Nitrogen Retention by Sheep. J. Animal Sci., **6**: 343. 1947.

(11) Loosli, J. K., and Harris, L. E. Methionine Increases the Value of Urea for Lambs. J. Animal Sci., **4**: 435. 1945.

(12) Lucas, H. L. A Method of Equalized Feeding for Studies with Dairy Cows. J. Dairy Sci., **26**: 1011. 1943.

(13) Morrison, E. B. *Feeds and Feeding*, 21st ed. The Morrison Publishing Co., Ithaca, N. Y. 1949.

(14) Owen, E. C., Smith, J. A. B., and Wright, N. C. Urea as a Partial Protein Substitute in the Feeding of Dairy Cattle. Biochem. J., **37**: 44. 1943.

(15) Rupel, I. W., Bohstedt, G., and Hart, E. B. The Comparative Value of Urea and Linseed Meal for Milk Production. J. Dairy Sci., **26**: 647. 1943.

(16) Snedecor, G. W. *Statistical Methods.* 4th ed. The Iowa State College Press. Ames. 1946.

(17) Swift, R. W., Thacker, E. J., Black, A., Bratzler, J. W., and James, W. H. Digestibility of Rations for Ruminants as Affected by Proportions of Nutrients. J. Animal Sci., **6**(4): 432. 1947.

(18) Smuts, D. B., and Marais, J. S. C. Plant Proteins. V. The Biological Value of Lucerne and Lucerne Supplemented by Cystine in Sheep. Onderstepoort. J. Vet. Sci. and Animal Inds., **11**: 399. 1938.

(19) Willett, E. L., Henke, L. A., and Maruyama, C. The Use of Urea in Rations for Cows under Hawaii Conditions. J. Dairy Sci., **29**: 629. 1946.

DIFFERENCES IN MILKING RESPONSE UNDER PRESCRIBED VARIATIONS IN METHODS EMPLOYED TO STIMULATE MILK LET-DOWN[1, 2]

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Massaging the udders of dairy cows prior to milking is widely advocated as a beneficial practice in stimulating milk let-down. Saveliev (11) reported a saving of 0.75 min. in average milking time when the udder was massaged before milking and at intervals during milking. Smith and Petersen (12) observed a faster rate of milk flow and a consequent saving in milking time where the udders were washed in warm water and each quarter was fore-milked before applying the milking machine. Knoop and Monroe (9), likewise, found that a pre-milking udder massage shortened the time of milking.

Although the use of warm water has been and still is being stressed as an important adjunct to udder massage in stimulating milk let-down, Dodd and Foot (3, 4) and Knoop and Monroe (9) found cold water to be just as effective. Homb *et al.* (6) obtained no differences in milk yields and rates of milk flow when the udders were massaged with a dry cloth as compared with a warm, wet cloth. Beck and Claydon (2) showed that massage with the dry, bare hands was just as effective in stimulating milk let-down as massage with a warm, damp cloth.

Based on the findings of Miller and Petersen (10), there should be little delay between the stimulation of milk let-down and the beginning of milking. They noted a reduction in milk yield when milking was delayed 20 min. after massaging the udder. This has been corroborated by Homb *et al.* (6) and by Ward and Smith (14). Homb *et al.* (6) also observed an increase in milking time when milking was delayed 15 min. after stimulation. Contrary to these results, Knodt *et al.* (7, 8) found no significant difference in milk yields for periods from 35 to 90 days in length when cows were milked at various intervals from 2 to 20 min. after stimulation.

Studies of the factors affecting the efficiency of machine milking and related problems were initiated at this Station in 1947. Since that time, results of investigations elsewhere which include research of a similar nature to some herein reported have been published. Any duplication would seem justified, however, because that which is duplicated fits into the over-all pattern of step by step

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[2] Some of the data herein reported are taken from a thesis presented by D. B. Roark for the degree, Master of Science, at Kansas State College and part of the data are taken from a thesis presented by G. H. Beck to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree, Doctor of Philosophy.

[3] Now at Mississippi State College.

evolvement of this complete study. Also, the technics and procedures are considered sufficiently different to be worthwhile.

EXPERIMENTAL PROCEDURE

Five different trials were conducted in which the pre-milking preparation of the cows was varied. Milking procedure was standardized by using the same milking machine unit, operated by the same person, at the same vacuum level and pulsation rate throughout each trial. The cows were milked at the same hour each milking, in the same stall and were fed grain just before milking. The rate of milk flow was measured graphically through the use of a specially adapted continuous-feed kymograph (1). Appropriate numerical measurements were taken from the milk flow curves in accordance with methods previously described (1). Variations in procedure followed in each trial are described below.

Trial I. Udder massage and fore-milking versus neither. A group of 16 cows, including four each of the Ayrshire, Guernsey, Holstein and Jersey breeds, were milked during three consecutive evenings when the cows were stimulated

Treatment	Water temperature	Length of preparation	Delay between preparation and milking
	(° *F.*)	(*sec.*)	(*min.*)
1	135	60	0
2	135	60	8
3	135	10	0
4	135	10	8
5	60	60	0
6	60	60	8
7	60	10	0
8	60	10	8
9	100	30	2

and three consecutive evenings when they were not. During the first three evenings, milk let-down was stimulated about 1 min. before milking by massaging the udder for 20 sec. with a cloth wrung out of warm water, then fore-milking each quarter.

Trial II. Milk let-down as affected by udder massage and/or fore-milking. Three different methods of stimulating milk let-down were employed, including (a) combination of udder massage, 20 sec. with a damp cloth and fore-milking, four full-hand squeezes from each quarter, (b) massage only and (c) fore-milking only. Fifteen cows selected at random from the college herd were divided into three groups of five each. During a 9-day period, each group was rotated through the series of three pre-milking treatments, with each method being used 3 days for each group.

Trial III. Combinations of udder wash water temperature, preparation time and time lapse between preparation and milking. This trial included nine different pre-milking treatments, as follows:

Eighteen cows were paired into nine groups of two each, and during an 18-day period each group was subjected to each treatment for a period of 2 days. The

order of rotation was determined by the use of a 9 × 9 Latin square from Fisher's tables (5).

Trial IV. Further variations in time lapse after preparation, with water temperature constant. Nine different pre-milking treatments were employed with 10-, 30- and 60-sec. preparation periods, and time lapses of 1, 3 and 5 min. between preparation and milking. Eighteen cows were divided into nine groups of two each, and during an 18-day period were rotated through the treatments in accordance with a 9 × 9 Latin square, as in Trial III.

Trial V. Effects of long-time (6 wk.) variations in time lapse with constant water temperature and preparation time. Cow groupings and pre-milking treatments were as follows:

Group I—6 cows—8-min. lapse
Group II—6 cows—5-min. lapse
Group III—4 cows—1-min. lapse

The cows were selected at random from a group that had been fresh at least 2 mo., but had not been bred more than 1 mo. Graphic milk flow measurements were recorded during the morning and evening milkings of five different 3-day periods, as follows: (a) pre-trial, (b) beginning of trial, (c) end of 3 wk., (d) last 3 days of the sixth week and (e) post-trial. During the pre- and post-trial measurements, all cows were milked under standard conditions with only a 1-min. delay between preparation and milking. Milk weights were recorded for each milking throughout the experiment.

RESULTS

In trial I, all cows milked out at a faster rate when their udders were massaged, and fore-milking was practiced before the milking machine was attached (table 1). Obviously milk let-down had been stimulated by this pre-milking

TABLE 1

Average response of cows to machine milking with and without pre-milking preparation

Pre-milking preparation	No. of cows	Total no. of milkings	Per cent of yield at 2 min.	Machine time (av./milking)	Milk yield (av./milking)	
					Obtained by machine	Hand stripping
				(*min.*)	(*lb.*)	(*lb.*)
Massage and fore-milking	16	48	77 **	3.2 **	14.3 **	0.2
None	16	48	53	4.4	12.7	0.7

** Differences significant at $P < 0.01$ level.

manipulation of the udder because milk flow started almost immediately after the machine was attached, whereas there was a lag of about 1 min. before a substantial flow of milk started when there had been no pre-milking preparation. An average of 77 per cent of the milk was obtained in the first 2 min. with pre-milking stimulation, as compared with only 53 per cent with no pre-milking stimulation. This pre-milking stimulation of milk let-down resulted in an aver-

age decrease of 1.2 min., or about 27 per cent less machine milking time. These results were statistically significant as tested by t test (13).

Statistically significant increases also were obtained in milk yields as a result of the pre-milking stimulus. There was an average increase of 1.6 lb. per milking in the milk obtained by machine where the udders had been massaged (table 1). Furthermore, 70 per cent less hand strippings were obtained, on the average, when milk let-down was stimulated before the machine was put on the cows.

In trial II, a combination of udder massage and fore-milking was more effective in stimulating milk let-down than either udder massage alone or fore-milking alone (table 2). Massage alone was the poorest method of the three. The aver-

TABLE 2

Relative effectiveness of massage, fore-milking, and a combination of massage and fore-milking in stimulating milk let-down as shown by the milking response

Pre-milking preparation	No. of cows	Total no. of milkings	Per cent of yield at 2 min.	Machine time (av./milking)	Milk yield (av./milking)	
					Obtained by machine	Hand stripping
				(*min.*)	(*lb.*)	(*lb.*)
Massage and fore-milking	15	45	78 *	3.11 *	16.6	0.7
Fore-milking only	15	45	76	3.25	16.1	0.9
Massage only	15	45	74 *	3.40 *	16.6	0.8

* Differences significant at $P < 0.05$ level.

age machine time when the cows were massaged and fore-milked was about 8 per cent faster than when massaged only, and 4 per cent faster than when fore-milked only. Differences in milking speed, as measured by per cent of the yield obtained in 2 min. and by actual machine time were statistically significant. No significant differences occurred in the total milk yields.

In Trial III, no statistically significant differences were obtained in the milking responses that could be attributed to temperature of the water used in cleaning and massaging the udders. The average speed of milk withdrawal and average milk yield were practically the same when water at 135° F. was used as compared with water at only 60° F. (table 3). No physical disturbances were noted which would indicate discomfort to the cows from either the warm or cold massage. This study was made during November when outside temperatures fluctuated between freezing and 70 to 80° F.; however, the barn temperature never dropped below about 50° F.

The length of time spent during the preparation period in massaging and fore-milking affected the milking response only slightly. Average milk yields were practically the same when the preparation period lasted 10 or 60 sec. (table 3). Statistically significant differences were obtained in the rates of milk flow, but the cows milked only about 5 per cent faster, which is not of practical importance. However, it was observed that the udders could not be cleaned properly in only 10 sec.

When milking was delayed 8 min. after stimulation of milk let-down, as compared with no delay, both the yields and rates of milk flow were decreased. The

TABLE 3

Average response of 18 cows to machine milking when temperature of the wash water, length of the massage period and length of the interval between massage and milking were varied

Pre-milking preparation	No. of milkings	Per cent of yield at 2 min.	Machine time (av./milking)	Milk yield (av./milking)	
				Obtained by machine	Hand stripping
			(*min.*)	(*lb.*)	(*lb.*)
Water temperature:					
135° F.	144	65	3.9	13.2	0.67
60° F.	144	65	3.9	13.4	0.72
Length of preparation:					
10 sec.	144	62	4.0	13.3	0.65
60 sec.	144	65 *	3.8 *	13.2	0.74
Delay in milking:					
0 min.	144	69	3.7	13.7	0.60
2 min.	36	70	3.6	13.6	0.63
8 min.	144	60 *	4.1 *	13.0 *	0.79

* Indicated difference significant at $P < 0.05$ level.

average machine yield was 5 per cent less and the average machine time was 14 per cent greater when milking was delayed 8 min. than when the standard 2-min. delay prevailed. Differences between a 2-min. delay and no delay were not statistically significant.

Results of the fourth trial were similar to those obtained in trial III with respect to length of the preparation period. Differences in milking speed, as shown by machine time and per cent of the milk yield obtained during the first 2 min. (table 4) were statistically significant when comparing 10-, 30- and 60-sec. periods; however, these differences were too small to be of practical importance. Differences in milk yields were not statistically significant.

In trial IV, a delay of 5 min. in milking caused a statistically significant increase in milking speed, as compared with delays of either 1 or 3 min. The increase in machine time was not so great, however, as was caused by the 8-min.

TABLE 4

Average response of 18 cows to machine milking when the length of massage period and length of the interval between massage and milking were varied

Pre-milking treatment	No. of milkings	Per cent of yield at 2 min.	Machine time (av./milking)	Milk yield (av./milking)	
				Obtained by machine	Hand stripping
			(*min.*)	(*lb.*)	(*lb.*)
Length of massage:					
10 sec.	108	65	3.7	11.2	0.55
30 sec.	108	67	3.6	11.3	0.49
60 sec.	108	68 *	3.5 *	11.2	0.52
Delay in milking:					
1 min.	108	69	3.5	11.1	0.52
3 min.	108	68	3.5	11.2	0.54
5 min.	108	64 *	3.7 *	11.3	0.49

* Differences among the above means were significant at $P < 0.05$ level.

delay in the third trial, in this case averaging only 6 per cent. Furthermore, the milk yields were not affected significantly.

In view of the relatively short duration of these experiments and the fact that there were frequent changes in milking routine, some doubt existed as to whether the changes in milking response were due to delayed milking or to the sudden changes in milking routine *per se*. Results of the fifth trial involving a 6-wk. period showed that there were no gross reductions in milk yields that could be attributed to delayed milking (table 5). The average milk yield obtained during each of the first 5 days at the beginning of the trial from the six cows in group I that were subjected to an 8-min. delay in milking amounted to 95, 96, 97, 98 and and 98 per cent of the pre-trial five-day average. This indicated a temporary drop in milk yield, probably the result of the change in milking routine, but the yield returned to almost the pre-trial level after the third day. The average production from group I was about 97 per cent of the pre-trial average at the end of 3 wk. and about 92 per cent during the sixth week (table 5). Similar results

TABLE 5

Effect of 5- and 8-min. delays in the start of milking on milk yields and milking time over a 6-wk. period

5-d. periods	Av. daily milk yields			Av. machine time/milking		
	Group I 8-min. delay	Group II 5-min. delay	Group III 1-min. delay	Group I 8-min. delay	Group II 5-min. delay	Group III 1-min. delay
	(*lb.*)	(*lb.*)	(*lb.*)	(*min.*)	(*min.*)	(*min.*)
Pre-trial	35.5	30.2	31.6	4.0	4.2	3.0
1st week	34.3	30.3	31.2	4.6	4.5	3.0
4th week	34.4	29.8	31.6	4.6	4.5	2.9
6th week	32.8	27.8	30.4	4.5	4.3	2.9
Post-trial	32.5	26.5	30.2	3.7	4.0	2.8

were obtained with the cows in group II, which were subjected to a 5-min. delay in milking. Although the average milk production of the control group (group III) decreased only 4 per cent during the 6-wk. period, the 8 per cent decrease in groups I and II does not appear to be abnormal.

The average milking time was increased significantly for groups I and II (table 5) as a result of delays in the start of milking. Slower rates of milking prevailed for both of these groups throughout the 6-wk. period, but immediately returned to normal when standard milking procedure was resumed. The fast-milking cows were affected more by delayed milking than were the slow-milking cows. For example, the three fastest milking cows in group I averaged 2.7 min. when milked under standard milking conditions as compared with 3.7 min. under delayed milking, or an increase of about 37 per cent, as compared with an average increase of only 15 per cent for all of the cows in this group.

The effect of an 8-min. delay in milking on the rate of milk flow from a fast- and a slow-milking cow is further illustrated in figure 1. The milk flow curves reproduced in this figure were obtained during a pre- and post-trial milking, and at the beginning, the middle and the end of the 6-wk. period. The graphs pre-

sented are typical of the response shown by other fast- and slow-milking cows used in this experiment. In B, C and D from cow 358, the milk flow was interrupted about 30 sec. after the machine was applied. The milk obtained prior to this point of interrupted flow can be accounted for by the fact that some milk normally is held in the teat and gland sinuses at the beginning of milking. The fact that milk flow did not continue at a constant rate, as is shown in curves A and E, indicates that the forces responsible for milk let-down were dissipated by the time the milking machine was applied. It also may be noted that the flow of milk was resumed after a lag of about 30 sec. This demonstrates that milk let-down was restimulated during the milking process, possibly by the action of the machine.

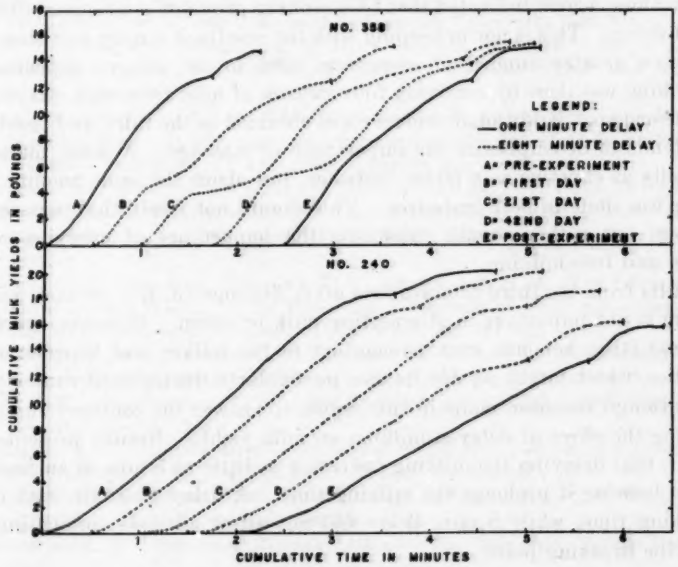

FIG. 1. Response of a fast- and a slow-milking cow to an 8-min. delay between stimulation of milk let-down and the start of milking.

There was no apparent disturbance in the milk flow of cow 240 (fig. 1) resulting from delayed milking. The reason for this difference in the response of fast- and slow-milking cows is obscure. Possibly a restimulation of milk let-down occurred in the slow-milking cows after milking started, but because of the slow rate of milk flow this was not reflected on the graph. From cow 358 (fig. 1) 4 to 5 lb. of milk were obtained before the milk flow was interrupted. This amount of milk was obtained in about 30 sec., whereas 60 to 70 sec. were required to obtain a similar amount of milk from cow 240. After 60 to 70 sec., milk let-down could have been restimulated, thus maintaining the constant rate of flow shown in the graphs for cow 240.

DISCUSSION

In agreement with other reports (9, 12) this investigation shows that mechanical milking is more efficient if milk let-down is stimulated before the milking machine is attached. Good dairy practice demands that the udder be cleaned prior to milking and the strip cup be used. Fortunately, this preparation of the cow to improve milk quality and to detect mastitis also stimulates milk let-down.

There probably are more dairymen who fail to use the strip cup than those who fail to clean at least partially, the udders by hand brushing. Results obtained in the foregoing experiments emphasize the importance of fore-milking in stimulating milk let-down. Fore-milking alone resulted in faster milking than massage alone, which indicated that fore-milking provided a stronger stimulus for milk let-down. This is not in keeping with the results of Knoop and Monroe (9); however, a greater number of cows were used in the present experiment and fore-milking was done by removing four streams of milk from each quarter rather than one or two. Additional evidence was obtained in the third and fourth trials, which tends to de-emphasize the importance of massage. A 5-sec. massage was practically as effective as a 50-sec. massage, but about the same amount of fore-milking was done in both instances. This should not imply that massage is unnecessary, but rather should emphasize the importance of carrying out both massage and fore-milking.

Results from the third trial confirm other findings (3, 4, 6, 9) that water temperature is not important in stimulating milk let-down. However, warm water may have other benefits, such as comfort to the milker and superior cleaning properties, which might justify its use, particularly during cold winter weather.

No attempt has been made in this report to answer the controversial question regarding the effect of delayed milking on milk yields. Results presented show, however, that delaying the milking operation as little as 5 min. is an undesirable practice because it prolongs the milking time. A delay of 3 min. had no effect on milking time, while 5 min. delay did not affect all cows, indicating that 4 min. is the breaking point.

SUMMARY AND CONCLUSIONS

Stimulation of milk let-down by massaging the udder with a damp cloth, then fore-milking each quarter of the udder shortened the milking time about 27 per cent, decreased the amount of hand strippings and increased milk yields.

Udder massage combined with fore-milking was more effective in stimulating milk let-down than either massage alone or fore-milking alone. Fore-milking only was more effective than massage only.

No significant differences were obtained in the milking responses of cows when their udders were massaged with damp cloths wrung out of water at 60, 100 or 135° F.

No more time need be spent in massaging the udder than is necessary for clean milk production.

Delaying the start of milking more than 3 or 4 min. after preparation of the cow is undesirable from the standpoint of prolonging the time required to milk.

REFERENCES

(1) Beck, G. H., Fryer, H. C., and Roark, D. B. Use and Interpretation of Milk Flow Curves in Measuring Variations in the Response of Cows to Machine Milking. J. Dairy Sci., **34**: 58–67. 1951.

(2) Beck, G. H., and Claydon, T. J. Relative Effectiveness of Paper Towels, Cloth Towels, and Dry Bare Hands in Cleaning the Udder and in Stimulating Milk Let-Down. J. Dairy Sci., **34**: 593–597. 1951.

(3) Dodd, F. H., and Foot, A. S. Experiments on Milking Technique. 1. Effect of Washing the Udder with Hot Water. 2. Effect of Reducing Milking Time. J. Dairy Research, **15**: 1–17. 1947.

(4) Dodd, F. H., and Foot, A. S. Experiments on Milking Technique. 3. Combined Effect of Reducing the Milking Time and Washing the Udder with Hot Water. 4. Effect of Increasing the Milking Time. J. Dairy Research, **16**: 14–22. 1949.

(5) Fisher, R. A. *Statistical Methods for Research Workers.* Oliver and Boyd, Edinburgh. 1949.

(6) Homb, T., Flatla, J. S., and Loe, B. Orienterende Forsok Med Ulike Metoder for Maskinmelking. (Preliminary Experiments on Different Methods of Milking by Machine.) Saertrykk, nr. **66**. 1948. (English summary.)

(7) Knodt, C. B., Reid, J. J., Williams, P. S., and Kesler, E. M. The Effect of the Interval between Washing of the Udder and Attachment of Milking Machines upon the Bacterial Flora and Milk Production of Dairy Cows. J. Dairy Sci., **30**: 580–581. 1947.

(8) Knodt, C. B., Reid, J. J., Williams, P. S., and Kesler, E. M. The Effect of the Interval between Washing of the Udder and Attachment of Milking Machines upon the Milk Production of Dairy Cows. J. Dairy Sci., **32**: 637–643. 1949.

(9) Knoop, C. E., and Monroe, C. F. Influence of Pre-milking Preparations of Cow's Udders upon the Let-down of Milk. J. Dairy Sci., **33**: 623–632. 1950.

(10) Miller, K., and Petersen, W. E. Some Factors Involved in Efficient Milking. J. Dairy Sci., **24**: 225–233. 1941.

(11) Saveliev, A. S. Machine Milking of Highly Productive Cows. Soviet Zootechnics, **4**: 67–77. 1941. (Abst., Dy. Sci. Abst.) 3: 149. 1941–1942.)

(12) Smith, V. R., and Petersen, W. E. The Effect of Preparation of the Cow on the Rate of Milking. J. Dairy Sci., **31**: 589–593. 1948.

(13) Snedecor, G. W. *Statistical Methods.* 4th ed. The Iowa State College Press, Ames. 1946.

(14) Ward, G. M., and Smith, V. R. Total Milk Production as Affected by Time of Milking after Application of a Conditioned Stimulus. J. Dairy Sci., **32**: 17–21. 1949.

ELECTROPHORETIC PROPERTIES OF MILK PROTEINS. I. PREPARATION OF SAMPLES OF SKIMMILK FOR ELECTROPHORETIC STUDIES

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In undertaking an electrophoretic study of skimmilk proteins by the method of Tiselius (9), it was necessary to establish the required technics. Therefore, the following procedures were investigated: preparation of skimmilk, storage properties of heated and unheated skimmilk and dialysis procedure.

Preparation of Skimmilk

Electrophoresis cannot be conducted satisfactorily on samples containing fat (3). Smith (8) suggested double separation in a centrifugal separator, but this procedure does not remove all ether-extractable material. It became necessary, therefore, to determine how much of this material could be tolerated in skimmilk without interference in electrophoresis. All fat determinations were made by the Mojonnier method.

Double separation in a conventional DeLaval centrifugal separator no. 514 of 400 lb. per hr. capacity yielded skimmilk testing from 0.051 to 0.070 per cent fat levels, which caused no difficulties in electrophoresis. Satisfactory skimming efficiency could be obtained by a single separation in a DeLaval airtight separator of 3,500 lb. per hr. capacity with the added advantage of economy of time. In all the runs made, the fat content was below 0.09 per cent, provided the first 25 gal. through the separator were not used.

The effect of fat in skimmilk is analogous to that observed by Longsworth (3) in blood serum. It makes a pattern appear as though a portion of it was overexposed and the other portion underexposed. The boundary between the two regions was found in skimmilk at pH 6.8 and 8.7 to be associated with component *2*, which was tentatively identified as β-lactoglobulin in subsequent studies. The light absorption due to fat became quite pronounced as the fat content increased above 0.1 per cent and in one instance where a sample tested 0.166 per cent fat an electrophoretic "schlieren" photograph could not be obtained. With a sample containing 0.12 per cent fat only the overexposed portion appeared on the film.

Storage Properties of Skimmilk

In order to establish the dialysis technic and the reproducibility of the electrophoretic patterns, it is desirable to employ skimmilk with as long a storage life as possible by the elimination of bacterial and enzymatic decomposition. Warner

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[1] The data published here are taken from a thesis presented by the senior author to the Faculty of the Graduate School, University of Illinois, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Feb., 1952.

(10) avoided this difficulty by addition of a preservative, but in this study it seemed desirable to accomplish this by heating to 300° F. by means of the Mallorizer (4).

Storage properties of heated skimmilk. The heating characteristics of the Mallory heater have been described previously by Herreid *et al.* (1). The milk as received at the University Creamery was separated at 85° F. and, after discarding the first 25 gal., the next 25 gal. of skimmilk were collected and pumped through the Mallorizer at the rate of 200 gal. per hour. The cycle was 4.0 sec. heating, 2.1 sec. holding and 6.1 sec. cooling. The heating temperature was determined by means of an iron-constantan thermocouple and a Rubicon portentiometer with the reference junction set at 32° F. The Mallorized skimmilk then was stored in an ice bath and at suitable time intervals samples were removed for electrophoresis.

A mixture of 10 ml. of the heated skimmilk and 20 ml. of Michaelis buffer (6) of pH 6.8 and $\mu = 0.173$ was dialyzed against 750 ml. of the same buffer at ice bath temperature for 48 hr., with a change of buffer after 24 hr. A mechanical dialyzer described by Reiner and Fenichel (7), using Visking seamless cellulose tubing as the membrane, was employed in these and all subsequent studies. Results of mobility measurements for all components are given in table 1. There were no significant changes in mobilities calculated from the false boundary attributable to storage and no new electrophoretically distinct components were formed during storage. As these data have been collected from the same sample of skimmilk, the results may be used as an indication of reproducibility of the electrophoretic procedure.

More constant values were obtained when the mobilities were measured from the false boundary, rather than from the initial boundary, which may have been due to a small shift of the entire column of protein suspension in the electrophoresis cell. The cause of the shift could not be determined, as all attempts to eliminate it failed to prevent it entirely. It may have been due to very slight leakage between the sections of the cell or to capillary forces in the two narrow channels of the cell, which might tend to delay attainment of hydrostatic equilibrium. The high degree of reproducibility of mobilities when measured from the false boundary suggests that no further disturbance of the pattern took place due to the shift and the mobilities so calculated are real and reproducible values.

A further test of reproducibility may be obtained from the results of table 2, which shows that changing the field strength did not affect the mobilities of raw skimmilk proteins in veronal buffer of pH 8.7 and 0.1 μ.

Storage properties of raw skimmilk. Fifteen ml. of a mixture of 5 ml. of skimmilk and 25 ml. of veronal buffer of pH 8.7 and 0.1 μ were dialyzed against the buffer for 24 hr. Raw skimmilk was stored in an ice bath and at suitable time intervals samples were removed for electrophoresis. Mobilities calculated from the false boundary are recorded in table 3.

A low protein concentration was employed in this study to avoid the formation of the flocculent precipitate which was observed after dialysis at higher concentrations, but it became apparent that it was too low for the best performance of

TABLE 1

Effect of storage at ice bath temperature on the mobilities of electrophoretic components of skimmilk Mallorized at 300° F. (A 1:3 dilution of skimmilk in Michaelis buffer at pH 6.8 and $\mu = 0.1$ after electrophoresis for 8,100 sec. at a field strength of 7.38 V./cm.)

Time of storage	Mobility after storage ($cm.^2\ sec.^{-1}\ V.^{-1} \times 10^5$)					
	Component 1		Component 2		Component 3	
	a[a]	d[a]	a	d	a	d
	Calculated from the initial boundary					
(d.)						
0	−2.48	−1.89	−3.29	−2.80	−4.69	−4.06
5	−2.84	−2.12	−3.65	−3.20	−4.96	−4.46
7	−2.70	−1.94	−3.52	−2.98	−4.87	−4.20
11	−3.20	−2.66	−4.06	−3.70	−5.32	−4.91
12	−2.66	−1.98	−3.43	−3.02	−4.82	−4.28
Mean	−2.78	−2.12	−3.59	−3.14	−4.93	−4.38
$\pm\hat{\sigma}$[b]	0.270	0.315	0.294	0.344	0.334	0.329
	Calculated from the false boundary					
0	−2.61	−1.80	−3.47	−2.70	−4.82	−3.97
5	−2.75	−1.71	−3.52	−2.80	−4.82	−4.06
7	−2.70	−1.76	−3.52	−2.79	−4.87	−4.01
11	−2.66	−1.80	−3.52	−2.84	−4.78	−3.88
12	−2.61	−1.67	−3.38	−2.70	−4.78	−3.97
Mean	−2.67	−1.75	−3.48	−2.77	−4.81	−3.98
$\pm\hat{\sigma}$	0.060	0.057	0.061	0.066	0.034	0.066
$\pm\hat{\sigma}$ all peaks[c]			0.0585			

[a] a = ascending, d = descending.
[b] $\hat{\sigma}$ = optimum standard deviation.
[c] calculated from the false boundary.

the electrophoresis apparatus and the boundaries appeared to lack the stability which they had at higher concentrations. Minor components were particularly difficult to interpret. A considerably higher optimum standard deviation was obtained for this series of runs, which may be due to the more erratic behavior at the lower protein concentration or to actual changes in mobility as the result of storage. In the absence of further confirmatory evidence, it was concluded that a two-day storage (*i.e.*, electrophoresis performed on the third day following collection of milk) did not change electrophoretic mobilities. The patterns for

TABLE 2

Effect of potential gradient on the mobilities of electrophoretic components of 1:6 diluted skimmilk at pH 8.7. (Veronal buffer, $\mu = 0.1$)

Potential gradient	Time of electrophoresis	Mobility ($cm.^2\ sec.^{-1}\ V.^{-1} \times 10^5$)			
		Component 0	Component 1	Component 2	Component 3
		d[a]	d	d	d
(V./cm.)	(sec.)				
11.72	4,500	−1.83	−3.11	−4.46	−5.98
5.63	9,000	−1.94	−3.16	−4.42	−6.02

[a] d = descending.

one and two days of storage were visually superimposable, indicating that no new components were formed. This is the most rigorous conclusion that could be drawn from these results, but justification may be found for it when it is remembered that milks of varying qualities should not be expected to keep for the same period of time. The skimmilk, after 12 days of storage at ice bath temperature, had a bacterial count of 900,000 per ml. at 10° C. incubation.

Dialysis Procedure

Reiner and Fenichel (7) concluded that a mixture of 10 ml. of blood serum and 20 ml. of veronal buffer reached dialysis equilibrium against 20 vol. of the buffer in 150 min. at 1.5° C. in a mechanical dialyzer with Visking seamless cellulose tubing as the membrane. Preliminary runs in this study indicated that these findings could not be reproduced with skimmilk and a re-evalution of the

TABLE 3

Effect of storage at ice bath temperature on the mobilities of electrophoretic components of raw skimmilk. (A 1:6 dilution of skimmilk in veronal buffer at pH 8.7 and $\mu = 0.1$ after electrophoresis for 4,500 sec. at a field strength of 11.97 V./cm.)

Time of storage	Mobility after storage ($cm.^2\ sec.^{-1}\ V.^{-1} \times 10^5$)							
	Component 0		Component 1		Component 2		Component 3	
	a[a]	d[a]	a	d	a	d[c]	a	d
(*d.*)								
1	−2.52	−2.05	−3.70	−3.35	−5.00	.....	−6.40	−6.05
2	−2.65	−2.00	−3.75	−3.22	−4.90	.....	−6.40	−5.95
3	−2.52	−2.05	−3.65	−3.20	−4.78	.....	−6.32	−5.80
5	−2.48	−1.80	−3.55	−3.10	−4.68	.....	−5.98	−5.60
7	−2.50	.....[d]	−3.70	−3.10	−4.75	.....	−6.35	−5.75
10	−2.68	−2.00	−3.85	−3.42	.....[d]	.....	−6.58	−6.10
Mean	−2.55	−1.98	−3.70	−3.23	−4.82	.....	−6.34	−5.88
$\pm\hat{\sigma}$[b]	0.111	0.104	0.102	0.131	0.106	.....	0.197	0.167

[a] a = ascending, d = descending.
[b] $\hat{\sigma}$ = optimum standard deviation.
[c] unable to calculate mobility due to extreme asymmetry of peak.
[d] obscured by exposure.

dialysis process became necessary. Experiments were set up to test the following: permeability of the membrane to protein nitrogen, time to reach dialysis equilibrium without buffer change at one pH, confirmation of this time at other pH levels and the effect of dialysis with and without buffer change.

Determination of permeability of Visking seamless cellulose tubing to protein nitrogen. Raw skimmilk was dialyzed against phosphate buffer of pH 6.97 at ice bath temperature for 24 hr. Total and nonprotein nitrogen were determined on 10- and 40-ml. aliquots of the buffer, respectively, before and after dialysis and protein nitrogen was calculated by difference. Nonprotein nitrogen was obtained by a modification of the method of Menefee *et al.* (5), which consists of dissolving 6 g. of trichloroacetic acid in approximately 4 ml. of H_2O, adding this solution to 40 ml. of the sample and making up to 50 ml. with H_2O. The mixture was shaken immediately and allowed to stand at room temperature for

1 hr. Following filtration nitrogen was determined on a 10-ml. aliquot of the filtrate by the method of Hetrick and Whitney (2) with a slight modification. It was found necessary to use 0.8 ml. of concentrated H_2SO_4 in the digestion of the buffer after dialysis and 0.5 ml. before dialysis. This makes it appear as though a true blank was not used, but, in the almost complete absence of organic matter, such as was the case in the buffer before dialysis, 0.8 ml. of acid affected the color of the Nessler-ammonia complex so as to give transmission values of over 100 per cent when compared to the color of a reagent blank containing only 0.5 ml. of acid. After dialysis the increased amount of H_2SO_4 had no deleterious effect, as apparently a sufficient amount of it was used up in digesting the organic matter and its concentration was lowered beyond the level at which it interfered.

Table 4 indicates that some protein nitrogen diffused through the membrane

TABLE 4

Permeability of Visking seamless cellulose tubing. (Results given are the means of duplicate determinations)

Fat test of whole milk (%)	3.282
Fat test of skimmilk (%)	0.079
Nitrogen content (%)	0.482
pH of buffer	6.97
Ionic strength of buffer	0.11
Quantity of buffer used in dialysis (*ml.*)	750
Quantity of milk used in dialysis (*ml.*)	30
Temperature of dialysis	ice bath
Time of dialysis (*hr.*)	24

Sample	1	2	Av.
Total nitrogen dialyzed (*mg. in 10 ml.*)	0.1588	0.1619	0.1604
Nonprotein nitrogen dialyzed (*mg. in 10 ml.*)	0.1470	0.1463	0.1467
Protein nitrogen dialyzed (*mg. in 10 ml.*)	0.0118	0.0156	0.0137
Protein nitrogen dialyzed in 750 ml. of buffer (*mg.*)	0.8850	1.1700	0.9825
Nitrogen in 30 ml. of skimmilk (*mg.*)	148.94	148.94	148.94
Protein nitrogen in 30 ml. of skimmilk (*mg.*)	137.47	137.53	137.50
Protein nitrogen dialyzed from skimmilk (%)	0.643	0.851	0.747

but, since the amount that diffused was less than 1 per cent of the total nitrogen in the milk, it constituted an amount which could not be detected with the electrophoresis apparatus used in this study. Therefore, Visking seamless cellulose tubing was considered a satifactory membrane for the dialysis of skimmilk.

Determination of dialysis equilibrium time. In checking the progress of dialysis, specific conductance and pH usually are determined. In this study three additional tests were employed: light absorption of the dialyzed suspension, the mobility of electrophoretic components and the area under the ϵ boundary. Dialysis was considered to be at equilibrium when pH, specific conductance, light absorption, mobilities and the area of the ϵ boundary remained constant within experimental error upon further dialysis.

Fifteen ml. of a mixture consisting of one part of skimmilk heated to 300° F. by means of the Mallorizer and two parts of Michaelis buffer of pH 6.8, 0.173 μ were dialyzed against 750 ml. of the same buffer at ice bath temperature in the mechanical dialyzer. Samples were dialyzed for 2, 8, 12, 15, 19, 24 and 36 hr.

Conductance measurements were made at 0° C. by means of an Industrial conductivity bridge and a Perkin-Elmer conductivity cell. Light transmission was measured in a Coleman spectrophotometer at 375 mμ. Since some of the suspensions were quite opaque, it was necessary to dilute all of them with five parts of the buffer. Measurements were made against water set at 100 per cent transmission. Electrophoresis was carried out for 8,100 sec. with a current of 18 ma. and mobilities were calculated from the false boundary. Areas of the false boundaries were measured with a planimeter on enlarged tracings, three of which were drawn for each boundary. The ε rather than δ boundary was selected for examination, as the phenomenon here was not complicated by the presence of a protein gradient.

pH measurements proved to be an unsatisfactory test as the dilution of the skimmilk with buffer was sufficient to equilibrate the pH. Results of all other measurements are summarized in figure 1. Only the mobilities of component *3d*

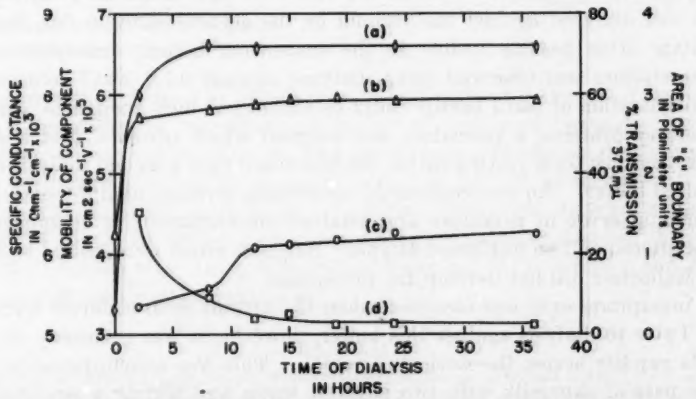
SPECIFIC CONDUCTANCE IN Ohm⁻¹ cm⁻¹ x 10³
MOBILITY OF COMPONENT IN cm² sec⁻¹ v⁻¹ x 10⁵
% TRANSMISSION 375 mμ
AREA OF "ε" BOUNDARY IN Planimeter units
(a)
(b)
(c)
(d)
TIME OF DIALYSIS IN HOURS

FIG. 1. Effect of time of dialysis on various properties of dialyzed skimmilk. A 1:3 dilution of skimmilk in Michaelis buffer at pH 6.8 and $\mu = 0.173$ at ice bath temperature. (a) Light transmission, (b) specific conductance, (c) mobility of component *3d*, (d) area of ε boundary.

were plotted because they were subject to the greatest variations and thus afforded the most rigorous test. Not all equilibria are established at the same time. Light transmission values did not change after 8 hr. of dialysis. Within an inherent error of ± 1 per cent in the conductance measurements this equilibrium was reached after 12 hr. of dialysis. This is a considerably longer time than that reported by Reiner and Fenichel (7) for blood serum. The difference may be due to a different behavior of the salt system in blood as compared to that in milk. The above authors stated that blood serum salts consist largely of NaCl and $NaHCO_3$. An even longer time for dialysis equilibrium is required from consideration of the mobilities and areas of false boundaries. It was shown earlier that the optimum standard deviation for the mobility of component *3d* was ± 0.066 and, therefore, at the 5 per cent confidence level 15 hr. or longer

were required for equilibrium. An optimum standard deviation for area measurements was found from eight separate tracings of a boundary with an approximate area of 0.5 planimeter units and was calculated to be 0.032. Therefore, at the 5 per cent confidence level this equilibrium was established after 19 hr. of dialysis.

On the basis of these findings a short dialysis time is not possible even with the use of the mechanical dialyzer, and therefore, a dialysis time of 24 hr. was selected for subsequent determinations because of the benefits of a safety factor and greater convenience of this time interval.

Confirmation of dialysis equilibrium time using other buffers. Fifteen ml. of a mixture of one part of raw skimmilk and three parts of buffer were dialyzed against three different buffers for 24 and 36 hr. The buffers used were veronal at pH 8.7, 0.1 μ, acetate at pH 5.6, 0.1 μ and glycine hydrochloride at pH 2.2, 0.1 μ.

In preliminary runs, it was observed that when raw skimmilk at this concentration was dialyzed against the veronal or the acetate buffer a fine flocculent precipitate often became visible as the suspension become transparent. The same precipitate was observed when dialyzed against 0.1*M* NaCl solution. As this concentration of NaCl hardly could be considered high enough to cause precipitation of proteins, a procedure was adopted which involved first a dialysis for 24 hr. against 0.1*M* NaCl solution, filtration and then a second dialysis against the desired buffer. An electrophoretic comparison revealed no differences within experimental error in mobilities and relative concentrations of components between a filtered and an unfiltered sample. Samples which were heated to 300° F. in the Mallorizer did not develop the precipitate.

No precipitate ever was observed when the glycine hydrochloride buffer was used. Prior to dialysis against this buffer, however, it was necessary to titrate the milk rapidly across the isoelectric region. This was accomplished by diluting one part of skimmilk with two parts of water and adding a predetermined amount of concentrated HCl rapidly under electric stirrer agitation. When this procedure was not followed, copious precipitation of proteins occurred. Results are given in tables 5, 6 and 7. Only component *Sa* in table 6 had a mobility after 36 hr. which was different by more than $2\hat{\sigma}$ ($\hat{\sigma}$ for all components = $\pm$ 0.0585) from that after 24 hr. of dialysis. All other components may be said with 95 per cent confidence to have remained unchanged under the two treatments. No explanation is available to account for the change in mobility of component *Sa*, but it was observed in other studies using only a 24-hr. dialysis period that the mobility of this component was not reproducible. At pH 5.6 no ϵ boundary was observed after 24 or 36 hr. of dialysis. It is possible, however, that it was present but could not be observed because of its small size and possible proximity of component *A*, whose counterpart was observed in the rising pattern, with the possible result of a raised base line and obscurement of both peaks. Since all differences in the areas of the ϵ boundary could be accounted for by the $2\hat{\sigma}$ spread, a conclusion that a 24-hr. dialysis under the above conditions is adequate appears justified.

TABLE 5

Effect of time of dialysis upon the electrophoretic properties of skimmilk diluted 1: 4 in veronal buffer of pH 8.7 and ionic strength of 0.1. (After electrophoresis for 4,500 sec. at a field strength of 11.98 V./cm.)

Component	Mobility after dialysis[a] for: ($cm.^2\ sec.^{-1}\ V.^{-1} \times 10^5$)	
	24 hr.	36 hr.
0a[b]	−2.91	−2.91
1a	−4.14	−4.14
2a	−5.65	−5.65
3a	−6.95	−6.95
0d[b]	−2.30	−2.30
1d	−3.32	−3.32
2d	−5.09	−5.01
3d	−6.29	−6.34
Area of ε boundary (*planimeter units*)	0.158	0.148

[a] Measured from the false boundary.
[b] a = ascending, d = descending.

Comparison of dialysis of skimmilk with and without a change of buffer. To secure a knowledge of the effects of double as compared to single dialysis, a direct comparison was made of the two procedures. In the single dialysis procedure, a mixture of 10 ml. of skimmilk heated to 300° F. by means of the Mallorizer and 20 ml. of Michaelis buffer of pH 6.8, 0.173 μ was dialyzed against the same buffer for 24 hr. at ice bath temperature. In double dialysis the outside buffer was replaced after 24 hr. with the same amount of fresh buffer and dialysis was continued for an additional 24 hr.

TABLE 6

Effect of time of dialysis upon the electrophoretic properties of skimmilk diluted 1: 4 in acetate buffer of pH 8.7 and ionic strength of 0.1. (After electrophoresis for 6,000 sec. at a field strength of 11.31 V./cm.)

Component	Mobility after dialysis[a] for: ($cm.^2\ sec.^{-1}\ V.^{-1} \times 10^5$)	
	24 hr.	36 hr.
Aa[b]	−0.75	−0.72
Ba	−1.24	−1.28
1a	−1.79	−1.83
Ca	−3.01	−3.01
Sa	−3.91	−4.20
3a	−5.22	−5.18
1d[b]	−1.34	−1.34
Cd	−2.73	−2.73
Dd	−4.53	−4.53
3d	−4.64	−4.64
Area of ε boundary (*planimeter units*)	0.00	0.00

[a] Measured from the false boundary in the ascending pattern and the initial boundary in the descending pattern.
[b] a = ascending, d = descending.

TABLE 7

Effect of time of dialysis upon the electrophoretic properties of skimmilk diluted 1:4 in glycine hydrochloride buffer of pH 2.2 and ionic strength of 0.1. (After electrophoresis for 5,400 sec. at a field strength of 8.49 V./cm.)

Component	Mobility after dialysis[a] for: ($cm.^2\ sec.^{-1}\ V.^{-1} \times 10^5$)	
	24 hr.	36 hr.
Ea[b]	2.52	2.52
Fa	4.84	4.84
Ga	6.14	6.22
Ha	7.69	7.65
Ja	9.62	9.62
Ed[b]	1.82	1.82
Fd	3.96	3.92
Gd	5.40	5.36
Hd	7.28	7.32
Jd	8.88	8.82
Area of ε boundary (*planimeter units*)	0.105	0.105

[a] Measured from the false boundary.
[b] a = ascending, d = descending.

Mobility measurements from all patterns are recorded in table 8 and it can be seen that with one exception all mobilities are somewhat higher from the double dialysis. Assuming an homogeneity of error, an analysis of variance calculated on the results of duplicate determinations revealed that double dialysis gave rise to different results which were significant at between the 5 per cent and the 1 per cent confidence level. If only single determinations are to be made, this advantage of double dialysis becomes somewhat questionable.

SUMMARY AND CONCLUSIONS

A satisfactory skimmilk for electrophoresis can be obtained by a single separation in a DeLaval airtight separator of 3,500 lb. per hour capacity.

TABLE 8

Effect of single and double dialysis on electrophoretic mobilities.[a] (1:3 dilution of skimmilk in Michaelis buffer of pH 6.8 and $\mu = 0.173$, after electrophoresis for 8,100 sec.)

Dialysis	Mobility ($cm.^2\ sec.^{-1}\ V.^{-1} \times 10^5$)					
	Component 1		Component 2		Component 3	
	a[b]	d[b]	a	d	a	d
Single[c]	−2.74	−1.97	−3.75	.....	−5.04	−4.28
Single	−2.86	−1.98	−3.83	−2.95	−5.15	−4.25
Mean	−2.80	−1.975	−3.79	−2.95	−5.095	−4.265
Double[d]	−2.93	−2.02	−3.81	−3.02	−5.17	−4.39
Double	−2.91	−2.07	−3.85	−3.11	−5.21	−4.37
Mean	−2.92	−2.045	−3.83	−3.065	−5.19	−4.38

[a] Measured from the false boundary.
[b] a = ascending, d = descending.
[c] Field strength = 7.52 V./cm.
[d] Field strength = 7.38 V./cm.

The presence of 0.09 per cent fat did not interfere in electrophoresis, but higher fat contents in skimmilk gave rise to light absorption and uneven exposure of photographic film. The boundary between the overexposed and underexposed portions of the patterns is found at the component *2* (β-lactoglobulin) boundary at pH 6.8 and 8.7.

Visking seamless cellulose tubing was found satisfactory as a dialysis membrane, as it allowed less than 1 per cent of the total protein nitrogen to dialyze out.

Skimmilk heated to 300° F. by means of the Mallorizer was stored for 14 days at ice-bath temperature, including dialysis, without showing change in its electrophoretic properties. Raw skimmilk stored for 3 days at ice-bath temperature, including dialysis, did not change electrophoretically.

It required between 15 and 19 hr. of dialysis with mechanical stirring to reach equilibrium as measured by mobilities and area of ϵ boundary. Specific conductance, light absorption and pH equilibria were established more rapidly.

Dialysis with a change of buffer gave rise to slightly higher mobilities than one without buffer change.

Dialysis for 24 hr. was found adequate in three different buffers having an ionic strength of 0.1 and pH's of 2.2, 5.6 and 8.7.

REFERENCES

(1) Herreid, E. O., Shahani, K. M., Dupuy, C. J., and Tracy, P. H. The Effect of Heating Cream in a Small-tube Heat Exchanger on Some of its Properties. J. Dairy Sci., 35: 13–20. 1952.

(2) Hetrick, J. H., and Whitney, R. M. Determination of Nitrogen in Milk by Direct Nesslerization of the Digested Sample. J. Dairy Sci., 32: 111–122. 1949.

(3) Longsworth, L. G., Curtis, R. M., and Pembroke, R. H., Jr. The Electrophoretic Analysis of Maternal and Fetal Plasmas and Sera. J. Clin. Invest., 24: 46–55. 1945.

(4) Mallory, W. E. U. S. Pat. no. 2,270,540. Official Gaz. U. S. Pat. Office, 34: 610. 1942.

(5) Menefee, S. G., Overman, O. R., and Tracy, P. H. The Effect of Processing on the Nitrogen Distribution in Milk. J. Dairy Sci., 24: 953–968. 1941.

(6) Michaelis, L. Der Acetat-Veronal-Puffer. Biochem. Z., 234: 139–141. 1931.

(7) Reiner, M., and Fenichel, R. L. Dialysis of Protein Solutions for Electrophoresis. Science, 108: 164–166. 1948.

(8) Smith, E. L. School of Medicine, University of Utah, Salt Lake City, Utah. Apr. 2, 1948. Personal communication.

(9) Tiselius, A. A New Apparatus for Electrophoretic Analysis of Colloidal Mixtures. Trans. Faraday Soc., 33: 524–531. 1937.

(10) Warner, R. C. Separation of α- and β-Casein. J. Am. Chem. Soc., 66: 1725–1731. 1944.

ELECTROPHORETIC PROPERTIES OF MILK PROTEINS. II. EFFECT OF HEATING TO 300° F. BY MEANS OF THE MALLORY SMALL-TUBE HEAT EXCHANGER[1] ON SKIMMILK PROTEINS

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Mellander (12) was the first to obtain an electrophoretic pattern of skimmilk and of casein. Other investigators (4, 5, 6, 7, 8, 14, 21) studied various preparations of casein. Slatter and Van Winkle (15) heated skimmilk to 65, 75 and 85° C. for 30 min. and observed changes in the electrophoretic patterns as a result of the heat treatment. The whey proteins of milk and colostrum have received considerable attention in the past few years (1, 2, 3, 7, 9, 11, 16, 17, 18, 19). While whey is considered to contain six or more components, only the immune globulins (16, 17) and β-lactoglobulin (1, 2, 9, 11, 18, 19) have been studied in detail.

The present research was undertaken to compare the electrophoretic properties of raw skimmilk with those of the same skimmilk heated at one well controlled heating temperature and time of exposure.

EXPERIMENTAL PROCEDURE

Milk as received at the University Creamery was separated and, after securing the unheated sample of skimmilk, the temperature of the balance was raised to 300° F. by means of the Mallory tubular heater. The cycle was 4 sec. heating, 2.1 sec. holding and 6.1 sec. cooling. The maximum variation in heating temperature was not greater than 1° F. Both unheated and heated samples were stored in an ice bath until dialysis.

The skimmilk was diluted to one-fourth the original concentration ($\sim$0.9 per cent protein) and dialyzed for 24 hr. against the following buffers: veronal at pH 8.7, acetate at pH 5.6 and glycine hydrochloride at pH 2.2. Electrophoresis was carried out in a Perkin-Elmer Tiselius electrophoresis apparatus for 4,500 sec. with a current of 12.0 ma. at pH 8.7, 6,000 sec. with a current of 12.5 ma. at pH 5.6 and 4,800 sec. with a current of 15 ma. at pH 2.2. Unless otherwise indicated, mobilities were calculated from the false boundaries of both the ascending and the descending patterns and area measurements were made from enlarged tracings by means of a planimeter. Corrections for the δ and ϵ effects were made as suggested by Longsworth *et al.* (10). Other details of methodology were given previously (20).

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[1] Mallorizer. U. S. Patent no. 2,270,540.

[2] The data published here are taken from a thesis presented by the senior author to the Faculty of the Graduate School, University of Illinois, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Feb., 1952.

RESULTS

Results showing the effect of heating to 300° F. by means of the Mallory heater are given in tables 1, 2 and 3 and in figure 1. The data represent the means of three determinations on different milk samples. With the exception of the mobility of one component at one pH, replicate determinations did not differ by more than twice the standard deviation from the mean as determined from five replicates for mobility measurements and six replicates for the area measurements obtained from a single milk sample (20).

TABLE 1

Effect of Mallorization of 300° F. on the electrophoretic properties of skimmilk proteins at pH 8.7. (1: 4 dilution of skimmilk equilibrated against veronal buffer of 0.1 ionic strength)

Component	Ascending			Descending		
	Absolute area	Relative area[a]	Mobility[b]	Absolute area	Relative area[a]	Mobility[b]
	(*planimeter units*)	(%)	(*cm.² sec.⁻¹ V.⁻¹ × 10⁵*)	(*planimeter units*)	(%)	(*cm.² sec.⁻¹ V.⁻¹ × 10⁵*)
			Unheated			
0 (γ-casein)	0.044	3.3	−2.92	0.035	2.4	−2.32
1 (β-casein)	0.384	29.5	−4.16	0.357	24.0	−3.34
2 (β-lactoglobulin)	0.160	12.2	−5.54	0.128	8.7	−5.09
3 (α-casein)	0.715	54.9	−6.95	0.970	64.8	−6.35
			Mallorized (300° F.)			
0 (γ-casein)	0.040	2.7	−2.98	0.022	1.5	−2.22
1 (β-casein)	0.420	28.7	−4.21	0.363	24.6	−3.39
2 (β-lactoglobulin)	0.132	9.0	−5.38	0.110	7.5	−4.69
3 (α-casein)	0.868	59.5	−6.82	0.982	66.5	−6.08

[a] Corrected for the δ and ε effect.
[b] Calculated from the false boundary.

DISCUSSION

Identification of components. Mellander (12), working with a phosphate buffer of pH 6.9 and 0.1 μ presented evidence that the electrophoretically observed components of skimmilk are α-casein, β-lactoglobulin, β-casein and γ-casein, in the order of decreasing mobilities. Although the boundary which Mellander considered to be due to γ-casein now is generally recognized as a false boundary, the existence of α- and β-casein has been confirmed by Krejci *et al.* (8) and Warner (21). A very similar pattern of skimmilk to that of Mellander was obtained by the present authors (20) in Michaelis buffer of pH 6.8, 0.173 μ.

Warner (21), postulating only two components of casein in veronal buffer of pH 7.78, 0.1 μ, reported the mobility of the α-casein boundary as -6.30×10^{-5}

and that of the β-casein boundary as -2.92×10^{-5}. Hipp *et al.* (4) observed three components of casein in veronal buffer of pH 8.4, 0.1 μ using a protein concentration of 1 per cent. Upon fractionation, these components yielded mobilities of -6.75×10^{-5} for α-casein, -3.05×10^{-5} for β-casein and -2.01×10^{-5} for γ-casein. The values obtained in the present study for raw skimmilk at pH 8.7, 0.1 μ calculated from the descending patterns were -6.35×10^{-5}, -5.09×10^{-5}, -3.34×10^{-5} and -2.32×10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$. Upon comparison with the results of Warner and Hipp *et al.*, the only component which does not agree within reasonable limits is the one with a mobility of -5.09×10^{-5}, and it may be assumed, therefore, that this component is not a casein fraction.

TABLE 2

Effect of Mallorisation at 300° F. on the electrophoretic properties of skimmilk proteins at pH 5.6. (1:4 dilution of skimmilk equilibrated against acetate buffer of 0.1 ionic strength)

Component	Ascending			Descending		
	Absolute area	Relative area[a]	Mobility[b]	Absolute area	Relative area[a]	Mobility[c]
	(*planimeter units*)	(%)	(*cm.^{2} sec.$^{-1}$ V.$^{-1} \times 10^5$*)	(*planimeter units*)	(%)	(*cm.^{2} sec.$^{-1}$ V.$^{-1} \times 10^5$*)
			Unheated			
A ($\beta - \gamma$-casein complex)	0.015	1.3	−0.75	None	None	None
B ($\beta - \gamma$-casein complex)	0.060	5.3	−1.27	None	None	None
1 (β-casein)	0.283	24.7	−1.77	0.370	27.6	−1.30
2 (β-lactoglobulin)	0.024	2.1	−3.27	0.021	1.6	−2.81
S	0.060	5.3	−4.20	None	None	None
T (α-casein- β-lactoglobulin complex)	None	None	None	0.071	5.3	−4.50
3 (α-casein)	0.695	61.2	−5.31	0.806	65.5	−4.80
			Mallorized (300° F.)			
A ($\beta-\gamma$ casein complex)	0.019	1.5	−0.76	None	None	None
1 (β-casein)	0.309	24.4	−1.77	0.290	19.9	−1.39
2 (β-lactoglobulin)	0.032	2.4	−3.38	0.045	3.1	−3.06
S	0.088	7.0	−4.20	None	None	None
3 (α-casein)	0.818	64.5	−5.20	1.118	76.9	−4.68

[a] Corrected for the δ and ε effect.
[b] Calculated from the false boundary.
[c] Calculated from the initial boundary.

Mobilities reported for β-lactoglobulin in veronal buffer of pH 8.6 and an ionic strength of 0.1 were -5.1×10^{-5} by Smith (16) in a mixture of whey proteins

and -5.8×10^{-5} by Bain and Deutsch (1) as a separated fraction. Since several investigators (12, 17, 21) have shown that the mobilities of milk proteins are not the same in mixtures as in pure form, it may be assumed that Smith and Bain and Deutsch had reference to the same component of whey and that component *2* in the present study is β-lactoglobulin.

It also is to be noted that according to Menefee *et al.* (13) "albumin" nitrogen constitutes 8 to 11 per cent of the total nitrogen of milk, which values are of the same order of magnitude as the relative areas due to component *2* as shown in table 1.

Although in reporting mobilities and relative concentrations of component *3* (α-casein) in table 1, it was assumed that this component was homogeneous at

TABLE 3

Effect of Mallorization at 300° F. on the electrophoretic properties of skimmilk proteins at pH 2.2. (1:4 dilution of skimmilk equilibrated against glycine hydrochloride buffer of 0.1 ionic strength)

Component	Ascending			Descending		
	Absolute area	Relative area[a]	Mobility[b]	Absolute area	Relative area[a]	Mobility[b]
	(*planimeter units*)	(%)	($cm.^2\ sec.^{-1}\ V.^{-1} \times 10^5$)	(*planimeter units*)	(%)	($cm.^2\ sec.^{-1}\ V.^{-1} \times 10^5$)
			Unheated			
D	0.058	4.2	+2.65	0.042	3.1	+1.95
E	0.153	11.0	+3.65	0.140	10.4	+2.90
G	0.992	72.4	+6.21	1.010	74.2	+5.56
H	0.046	3.4	+7.86	0.057	4.3	+7.45
2 (β-lactoglobulin)	0.122	8.9	+9.87	0.108	8.0	+9.08
			Mallorized (300° F.)			
E	0.147	11.0	+3.70	0.132	9.3	+2.72
G	1.144	85.9	+6.19	1.191	87.6	+5.35
H	0.028	2.1	+7.80	0.036	2.5	+7.26
2 (β-lactoglobulin)	0.014	1.0	+9.61	0.010	0.7	+9.36

[a] Corrected for the δ and ε effect.
[b] Calculated from the false boundary.

pH 8.7, it can be seen from figure 1a that in the rising pattern of skimmilk component *3* split into three components. This is a confirmation of the results of Warner (21), who found that at a concentration of less than 1 per cent protein the rising boundary of α-casein was not homogeneous and lends further support to the assumption that component *3* in the present study was α-casein. Hipp *et al.* (5), in a recent publication, did not observe the splitting of the α boundary and ascribed that to the fact that a composite milk was used. In this study the inhomogeneity was observed even though a composite sample was employed.

It also was noted that, in some skimmilk samples, the three components of the rising boundary of α-casein were quite sharp and distinct, whereas in other samples one of the peaks was questionable and exhibited itself at most as an

asymmetry. Although this problem was not studied exhaustively, a possibility is suggested for differences in milks.

The relative area due to β-casein was larger in the ascending pattern than in the descending pattern. This also was observed by Krejci *et al.* (8) and by Warner (21) and was attributed by them, though not entirely explained, to an association phenomenon between α- and β-casein.

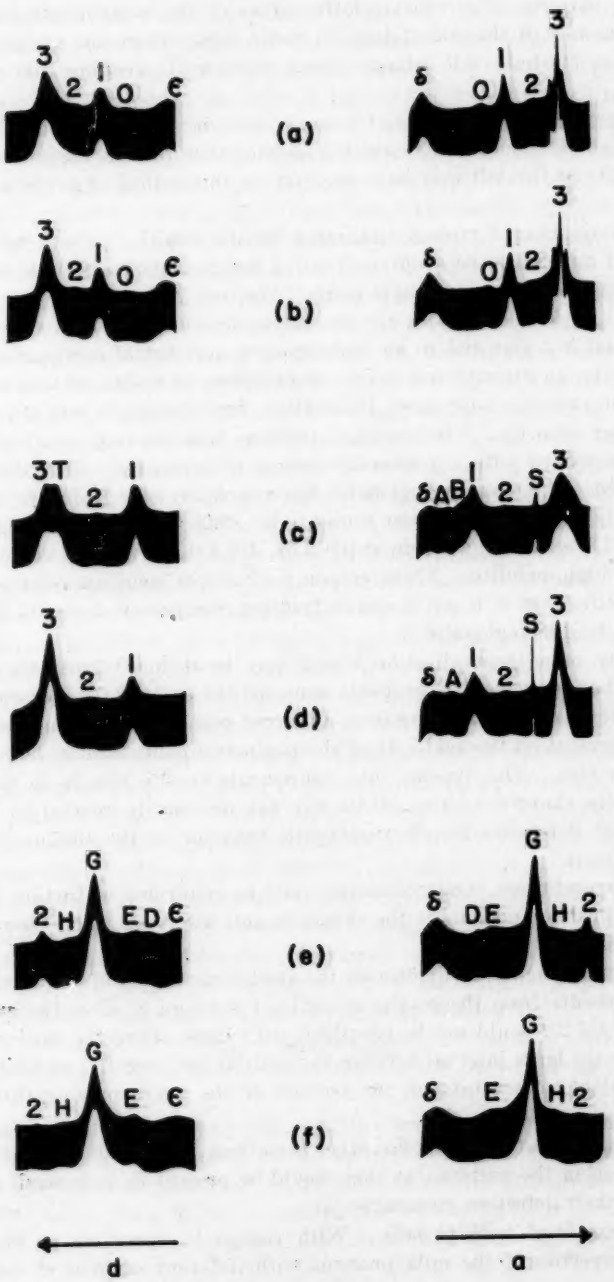
An examination of table 2 (unheated sample at pH 5.6) reveals considerable difference between the ascending and the descending patterns (also see figure 1c). While six components were present in the ascending patterns, there were only four in the descending patterns. Components *1* and *3* in both patterns appeared to have remained similar to those observed at pH 8.7, but even they have lost considerable sharpness which suggests some inhomogeneity. Component *S* was observed by Warner in a system containing pure α- or β-casein and therefore it must be considered either an anomaly or an unknown casein fraction which is not resolved at higher pH.

γ-Casein, according to Hipp *et al.* (4), has an isoelectric point of pH 5.8 to 6.0 and should, therefore, have a positive mobility at pH 5.6. If that were so, γ-casein would not appear in any of the patterns and the relative area of the remaining components should increase by a proportional amount. Results of table 2 do not confirm such a hypothesis but rather lend support to a view that γ-casein still is moving with a negative mobility but that it is associated with β-casein. Therefore, components *A* and *B* may be γ-casein-β-casein complexes. In the descending patterns, these complexes are only exhibited as asymmetries of component *1* (β-casein).

According to Li (9) β-lactoglobulin is homogeneous at pH 5.6 in acetate buffer of an ionic strength of 0.1 and has a mobility of -2.5×10^{-5}. The bovine lactoglobulin of Bain and Deutsch had a mobility of -3.5×10^{-5} at pH 6.0 in acetate buffer with an ionic strength of 0.1. In the present study, a component with a mobility of -2.81×10^{-5} was observed at pH 5.6 but the relative area due to this component was not sufficient to account for all of β-lactoglobulin found in the patterns of pH 8.7 (component *2*).

Examination of the electrophoretic patterns of casein obtained by Warner (21) at pH 5.63 discloses homogeneous α- and β-casein boundaries and it suggests, therefore, that the inhomogeneity of the patterns obtained in the present study was caused by inhomogeneity of these components of skimmilk. The relative areas of all components found at pH 8.7 (table 1) may be accounted for at pH 5.6 (table 2) by assuming a complex formation between γ- and β-casein as shown above and another complex between β-lactoglobulin and α-casein (component *T*), which is present only as an asymmetry of component *1* (α-casein complex) in

FIG. 1. Effect of heating to 300° F. with a Mallorizer on the electrophoretic patterns of 1 to 4 dilution of skimmilk in various buffers of $\mu = 0.1$. (a) Unheated and (b) heated skimmilk in veronal buffer of pH 8.7 after 4,500 sec. electrophoresis with a current of 12 ma. (c) Unheated and (d) heated skimmilk in acetate buffer of pH 5.6 after 6,000 sec. electrophoresis with a current of 12.5 ma. (e) Unheated and (f) heated skimmilk in glycine hydrochloride buffer of pH 2.2 after 4,800 sec. electrophoresis with a current of 15 ma.


3
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the rising pattern. The exact relative areas of the components could not be obtained because of the uncertainty, in many cases, where one component ended and another started. All interpretations were made keeping this uncertainty in mind.

No association between α- and β-casein need be postulated at pH 5.6 to account for all the components, which indicates that the association observed by Warner (21) at this pH may have been due to the method of preparation of the casein.

An examination of table 3 (unheated sample at pH 2.2) and figure 1d discloses what appears to be a different set of components from those observed on the alkaline side of the isoelectric point. Warner (21) and Hipp *et al.* (4) reported that α-, β- and γ-casein are not homogeneous on the acid side of the isoelectric point but give rise to an undetermined number of components. In the present study, an attempt was made, nevertheless, to assign certain well-defined areas of the patterns component designation, even though it was not possible to deduce their identity. Component *2* perhaps was the only component which could be identified with a reasonable degree of certainty. According to Bain and Deutsch (1), bovine lactoglobulin has a mobility of $+10.3 \times 10^{-5}$ at pH 3.0. The mobility of component *2* was found to be $+9.08 \times 10^{-5}$ (descending pattern). Warner (21), in studying casein at pH 3.07, did not observe any component that had such a high mobility. Therefore, on the basis of mobility, relative area and the probability that it is not a casein fraction, component *2* at pH 2.2 may be assumed to be β-lactoglobulin.

The only other generalization which may be deduced from the results at pH 2.2 is that component *G* probably contains the bulk of the α-casein. It also should be pointed out that there is no apparent reason why the same components should be present on the acid side of the isoelectric point as have been found on the alkaline side. The division into components on this side is on the basis of the positively charged groups, which are not necessarily related to the acidic groups which determine the electrophoretic behavior on the alkaline side of the isoelectric point.

The interpretations rendered above could be confirmed in further studies by adding purified components to the skimmilk and observing where they appeared in the patterns.

No effort was made to determine the isoelectric points of the various skimmilk constituents from the results of tables 1 through 3, since the casein components at pH 2.2 could not be identified with those above the isoelectric point and because the large interval between the positive and negative mobilities placed a great deal of uncertainty on the portion of the curve passing through zero mobility.

It is unlikely that milk proteins other than those postulated above should have been observed in the patterns, as they would be present in such small quantities as to make their detection impossible.

Variations from milk to milk. With regard to variations in the electrophoretic properties of the milk proteins with different samples of milk, a rig-

orous statistical analysis would have failed because of the inhomogeneity of the errors in mobility determinations and a questionable homogeneity of error in area measurements. To get some indication, however, of the constancy of the milk a homogeneous error was assumed and the $2\hat{\sigma}$ test was applied to the mobilities and relative areas of all components. The differences in the relative areas of all components in the various milks were less than the $2\hat{\sigma}$ spread and only the mobility of component *S* at pH 5.6 in the sample Mallorized at 300° F. was significant at the 5 per cent level. It was noted, however, in the present study as well as in previous runs at pH 5.6 that the mobility of this component was not reproducible in the same milk as well as between different milks.

Effect of heating by means of the Mallory heater at 300° F. The effect of Mallorization at 300° F. on the electrophoretic properties of skimmilk at pH 8.7 may be observed from table 1 and figure 1b. The mobilities of components *2* (β-lactoglobulin) and *3* (α-casein) were lowered by heating but a greater decrease was observed in the descending than in the rising patterns suggesting that heating resulted in greater viscosity of the skimmilk. However, this explanation alone is not tenable because components *0* (γ-casein) and *1* (β-casein) in the rising patterns should have been slowed by the greater viscosity also, which is contrary to the results. It may be speculated, therefore, that Mallorization at 300° F. in some way changed the particle structure so as to alter the available negative groups of components *2* (β-lactoglobulin) and *3* (α-casein). β- and γ-casein appear to be more heat stable in this respect.

Mallorization at 300° F. also resulted in greater symmetry of component *3* (α-casein) (figure 1b) which also may have been due to a change in structure with the result of making more uniform the constituents of that component.

Examination of table 2 and figure 1d (pH 5.6) reveals that Mallorization at 300° F. resulted in the disappearance of component *B* (β-, γ-casein complex) in the ascending pattern and component *T* (α-casein-β-lactoglobulin complex) in the descending pattern while the relative area of component *3* (α-casein complex) increased, apparently at the expense of components *B* and *T*. This implies that α-casein in the sample Mallorized at 300° F. is a complex of α-, β- and γ-casein and β-lactoglobulin. It is interesting to note that this interpretation requires that the only β-casein that is complexed with α-casein is that which was originally complexed with γ-casein in the unheated sample.

The fact that the Mallorized sample at pH 5.6 exhibited α-, β-casein association is a qualitative confirmation of the findings of Warner (21). The absence of the phenomenon in the unheated samples suggests that the association was induced by heat or in Warner's work by the method by which the casein was prepared.

The mobility of α-casein (complex) at pH 5.6 was lowered by Mallorization at 300° F. from that of the α-casein in the unheated sample, while that of component *2* which may be postulated as the uncomplexed β-lactoglobulin was increased. The latter phenomenon is reminiscent of the findings of Briggs and Hull (2) that β-lactoglobulin upon heat denaturation gave rise to a peak of higher

mobility. The mobility of β-casein and component *A* remained unchanged, in which respect both γ- and β-casein behaved in the same manner as at pH 8.7.

An examination of figure 1b discloses that the boundaries due to component *1* (β-casein) and *3* (α-casein complex) are much sharper in the samples Mallorized at 300° F. than in the unheated samples and exhibit very little, if any, inhomogeneity, indicating at least for α-casein that a change of structure may have taken place.

At pH 2.2, as may be seen in table 3 and figure 1e, Mallorization at 300° F. resulted in a redistribution of component *2* (β-lactoglobulin) and component *D*. Component *G* showed a marked increase in relative area apparently at the expense of β-lactoglobulin and component *D*.

The mobility of β-lactoglobulin upon heating increased in the descending pattern and decreased in the ascending pattern. This may have its explanation in the fact that due to the extremely small size of this component its velocity was difficult to measure. All other components exhibited lower mobilities in the descending pattern upon Mallorization while no change in the mobilities was observed in the ascending pattern. It was observed at all pH levels that the mobilities calculated from the ascending patterns were less sensitive to change upon heating than were those calculated from the descending patterns.

CONCLUSIONS

On the basis of studies conducted with 1:4 dilution of skimmilk in veronal (pH 8.7), acetate (pH 5.6) and glycine hydrochloride (pH 2.2) buffers with an ionic strength of 0.1, the tentative identification of electrophoretic components in the order of increasing mobilities is as follows:

(a) at pH 8.7: γ-casein, β-casein, β-lactoglobulin and α-casein.

(b) at pH 5.6: β-, γ-casein complex *A*, β-, γ-casein complex *B*, β-casein, β-lactoglobulin, component *S* which is either an unknown casein component or an anomaly appearing only in the ascending pattern, α-casein-β-lactoglobulin complex *T* and α-casein.

(c) at pH 2.2: *D*, *E*, *G* and *H* are unidentified casein components, and the fifth component is β-lactoglobulin.

The following changes are indicated in the electrophoretic properties as a result of heating to 300° F. by means of the Mallory heater:

(a) at pH 8.7: the mobilities of α-casein and β-lactoglobulin are decreased, possibly due to structural changes, and the boundary of α-casein becomes more symmetrical.

(b) at pH 5.6: the β-, γ-casein complex *B* becomes associated with the α-casein-β-lactoglobulin complex and free α-casein to form a more or less electrophoretically homogeneous component with a mobility lower than that of original α-casein, while the mobility of free β-lactoglobulin is increased.

(c) at pH 2.2: β-lactoglobulin and the unidentified casein component *D* are redistributed among the other components with the mobilities of all casein components apparently lowered.

REFERENCES

(1) Bain, J. A., and Deutsch, H. F. Studies on Lactoglobulin. Arch. Biochem., 16: 221–229. 1948.

(2) Briggs, D. R., and Hull, R. Protein Denaturation. I. Electrophoretic Study of the Kinetics at Neutrality of the Heat Denaturation of β-Lactoglobulin. J. Am. Chem. Soc., 67: 2007–2014. 1943.

(3) Deutsch, H. F. A Study of Whey Proteins from the Milk of Various Animals. J. Biol. Chem., 169: 437–448. 1947.

(4) Hipp, N. J., Groves, M. L., Custer, J. H., and McMeekin, T. L. Separation of γ Casein. J. Am. Chem. Soc., 72: 4928–4931. 1950.

(5) Hipp, N. J., Groves, M. L., Custer, J. H., and McMeekin, T. L. Separation of α-, β- and γ-Casein. J. Dairy Sci., 35: 272–281. 1952.

(6) Kamal, S., and Turner, C. W. Electrophoretic Studies of Commercial and Iodinated Casein. J. Dairy Sci., 34: 790–797. 1951.

(7) Kemp, A. R. Ph.D. Thesis. University of Wisconsin. 1951.

(8) Krejci, E. L., Jennings, R. K., and Smith, L. DeS. Electrophoretic Evidence for Complex Formation in Casein. J. Franklin Inst., 232: 592–596. 1941.

(9) Li, C. H. Electrophoretic Inhomogeneity of Crystalline β-Lactoglobulin. J. Am. Chem. Soc., 68: 2746–2747. 1946.

(10) Longsworth, L. C., Curtis, R. M., and Pembroke, R. H., Jr. The Electrophoretic Analysis of Maternal and Fetal Plasmas and Sera. J. Clin. Invest., 24: 46–55. 1945.

(11) McMeekin, T. L., and Dellamonica, E. Separation and Properties of Bovine Whey Proteins. Federation Proceedings, 6: 1. 1947.

(12) Mellander, O. Electrophoretische Untersuchung von Casein. Biochem. Z., 300: 240–245. 1939.

(13) Menefee, S. G., Overman, O. R., and Tracy, P. H. The Effect of Processing on the Nitrogen Distribution in Milk. J. Dairy Sci., 24: 953–968. 1941.

(14) Nitschmann, H., and Lehmann, W. Electrophoretic Differentiation between Acid Casein and Labcasein. Experientia, 3: 153–154. 1947.

(15) Slatter, W. L., and Van Winkle, Q. Preliminary Observations on the Electrophoresis Study of the Proteins in Skimmilk. J. Dairy Sci., 33: 397. 1950.

(16) Smith, E. L. Bovine Immune Proteins. J. Biol. Chem., 164: 345–357. 1945.

(17) Smith, E. L. Isolation and Properties of Immune Lactoglobulins from Bovine Whey. J. Biol. Chem., 165: 665–676. 1946.

(18) Stanley, W. G., Andrews, A. C., and Whitnah, C. H. Electrophoresis of Milk Proteins. I. Some Comparisons of Salt-acid and Salt-lyophilized Whey Fractions. J. Dairy Sci., 33: 275–280. 1950.

(19) Stanley, W. G., Whitnah, C. H., and Andrews, A. C. Electrophoresis of Milk Proteins. II. Some Effects of Methods of Preparation on the Electrophoretic Patterns of Whey Proteins. J. Dairy Sci., 34: 265–272. 1951.

(20) Tobias, J., Whitney, R. M., and Tracy, P. H. Electrophoresis of Milk Proteins. I. Preparation of Samples of Skimmilk for Electrophoretic Studies. J. Dairy Sci., 35: 1026–1035. 1952.

(21) Warner, R. C. Separation of α- and β-Casein. J. Am. Chem. Soc., 66: 1725–1731. 1944.

LIPIDS IN BLOOD PLASMA OF YOUNG DAIRY CALVES[1, 2]

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The lipid level in the blood plasma of the newborn dairy calf has been demonstrated to be extremely low and marked increases occur after consumption of colostrum (2, 18). Although the nutritional implications are not clearly understood, this change may involve an increase in certain plasma lipid fractions essential to normal post-natal development. The lipid portion of colostrum and whole milk is an important dietary source of energy and of certain fat-soluble vitamins for the young calf, but whether the lipids supply other essential nutrients is unknown.

Since little information is available regarding the various plasma lipids of the young calf, the present investigation was initiated to study the early post-natal changes in the blood plasma levels of neutral fats, phospholipids, free cholesterol, ester cholesterol, "ester" fatty acids and "free" fatty acids.

EXPERIMENTAL

Seven Holstein and four Brown Swiss calves from the Iowa State College dairy herd were allowed colostrum *ad libitum* from their respective dams for 3 days following birth, and were fed whole milk (5 lb. per 100 lb. body weight) at approximately 6 a.m. the fourth day.

Samples of venous blood were drawn at birth and on the second and fourth days thereafter (six calves) or at daily intervals during the first 4 days following birth (five calves). Except for the sampling at birth, all samples were collected between 9 and 10 a.m. Heparin was employed as the anticoagulant.

Following centrifugation of the blood, the lipids of each plasma sample were extracted with alcohol-ether (3:1) using a solvent: plasma ratio of 20 to 1. The flask containing the alcohol-ether extract was placed in a water bath maintained at 55 to 60° C. and the solution was evaporated to dryness under reduced pressure. The residue was extracted with redistilled Skellysolve A (petroleum ether, b.p. 32 to 36° C.) and the remaining portion was dissolved in water.

The Skellysolve A extract and the aqueous phase (made alkaline to phenol red if not initially alkaline) were combined and agitated to assure removal of all suspended material in the petroleum ether extract (19). After separation of the liquid phases, the petroleum ether layer was transferred to a volumetric flask. Three subsequent extractions of the aqueous phase were made with redistilled Skellysolve A. These petroleum ether extracts were combined and hereafter are referred to as solution A. The aqueous solution, which contained the "soaps" of the free fatty acids, was acidified with 25 per cent H_2SO_4 and the liberated "free" fatty acids were extracted with redistilled Skellysolve A.

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Aliquots of solution A were taken for the separation of the phospholipids with acetone and $MgCl_2$ (5). Free cholesterol was precipitated from the acetone soluble fraction by employing the digitonin procedure described by Boyd (6).

Aliquots of solution A were saponified according to the method of Boyd (6), and after acidification, the liberated fatty acids, cholesterol and nonsaponifiable components were extracted with redistilled Skellysolve A. The ester fatty acids (derived from the triglycerides, phospholipids and cholesterol esters) were separated from the mixture by employing an alcoholic-alkaline wash, as suggested by Wilson and Hansen (17). Total cholesterol was precipitated with digitonin (6).

The quantities of each of the above lipid fractions (free fatty acids, phospholipids, free cholesterol, ester fatty acids, and total cholesterol) were determined by the micro-oxidative techniques of Bloor (5) and Boyd (6). Neutral fat fatty acids were obtained by subtracting the sum of the phospholipid and cholesterol ester fatty acids from the total ester fatty acids; neutral fat was derived by multiplication of the neutral fat fatty acid values by 1.05 (6); and total lipids were obtained by summation of the appropriate plasma lipids (including the free fatty acids). In addition, the Allen volumetric method (1) was employed to estimate the plasma fat value for each sample.

TABLE 1

Blood plasma lipids in young dairy calves

Plasma lipid	Age (*days*)		
	0 (birth)	2	4
		(*mg./100 ml. plasma*)[a]	
Total lipid	59 ± 6	132 ± 12	151 ± 13
Ester fatty acids	34 ± 4	75 ± 9	82 ± 9
Neutral fats	23 ± 3	38 ± 6	37 ± 7
Ester cholesterol	11 ± 2	32 ± 2	38 ± 6
Free cholesterol	5 ± 1	7 ± 1	10 ± 1
Phospholipid	7 ± 3	29 ± 5	31 ± 5
Free fatty acids	8 ± 3	8 ± 2	10 ± 2
Allen fat	25 ± 3	96 ± 11	111 ± 11

[a] Mean values for 11 calves ± standard errors.

RESULTS

The mean blood plasma levels of the various lipids at 0, 2 and 4 days of age are presented in table 1. A comparison of the plasma lipids from the 2-day-old calves with those of the same animals at birth revealed marked rises in the levels of total lipids, ester fatty acids, ester cholesterol, phospholipids and Allen fat; a lesser increase in neutral fats; and no appreciable changes in free fatty acids and free cholesterol. The values for the samples drawn at 4 days of age were, in most cases, not appreciably different from those of the 2-day samples.

Since it became apparent during the early phase of this experiment that the consumption of colostrum resulted in rapid increases in certain of the plasma lipids, more frequent blood sampling and analyses to determine the daily changes seemed desirable. Thus, blood samples were drawn at daily intervals from the

last five calves employed in the experiment. The data for these calves, three Holsteins and two Brown Swiss, are presented in figures 1 and 2. The Allen fat levels and the values for the various lipid fractions, except the free fatty acids, attained maxima when the calves were 3 days of age. In general, the values on the fourth day tended to decline somewhat, probably due at least in part to the dietary change at this time. A comparison of the Allen fat values with the total lipids exclusive of the free fatty acids and phospholipids is presented in table 2. Except for the values for the calves at birth, the Allen fat levels and the adjusted lipid values were similar, and when the data for the various ages were pooled, the correlation between the two was 0.955. This high correlation is in agreement with the results of others (9, 13).

TABLE 2

Comparison of the Allen lipid values with the adjusted lipid values (total lipids minus the sum of the phospholipids and free fatty acids)

Age of calves	Allen lipids	Adjusted lipids
(*d.*)	(*mg./100 ml. plasma*)[a]	
0 (birth)	20 ± 5	37 ± 7
1	65 ± 15	65 ± 10
2	95 ± 6	95 ± 5
3	132 ± 16	120 ± 9
4	121 ± 11	113 ± 13

[a] Mean values for 5 calves ± standard errors.

DISCUSSION

Values for the plasma lipid fractions which have been reported in the literature indicate marked variations within species. Although it is probable that a number of factors have contributed to this variability, the great variety of analytical methods employed undoubtedly is of major importance. Since the oxidative micro-technique is readily adapted to the estimation of the various plasma lipid fractions and has been employed successfully in studies of these components in various species (5, 6, 15, 16, 20, 21), this procedure was selected for the present investigation.

The levels of the free fatty acids observed in young calves (table 1 and fig. 2) were comparatively low and apparently were not correlated closely with those of the other lipids. These values were similar, however, to those found in the mature bovine (8, 12, 21). Bloor (3) has indicated that the amount of free fatty acids in blood plasma may vary greatly and apparently is not related to the amount of total lipid. The origin and role of these acids, which sometimes are referred to as soaps, have not been fully elucidated. It has been suggested, however, that the free fatty acids may result from the breakdown of phospholipids during the extraction procedure (4, 12). In addition, it may be postulated that the free fatty acids could originate in part from the partial degradation of phospholipids due to the action of plasma lecithinases which have been found in the blood (19).

The values for the plasma phospholipids of dairy calves at birth were lower

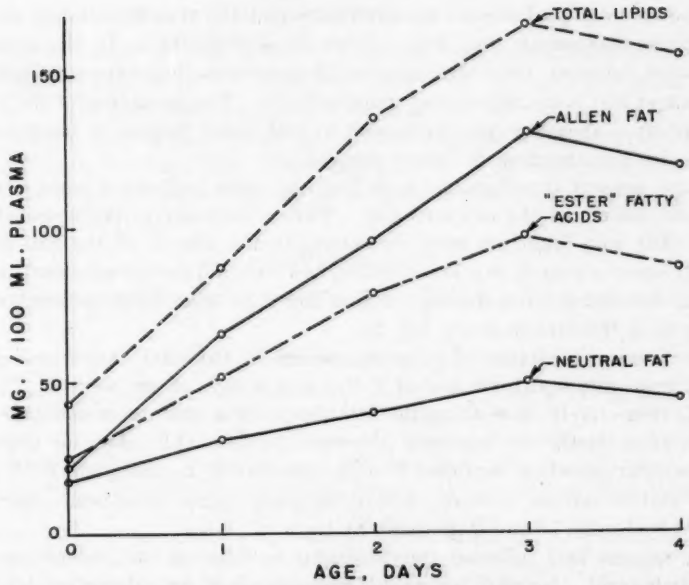

FIG. 1. Early post-natal changes, at daily intervals, in the mean total lipid, Allen fat, neutral fat and "ester" fatty acid values of the blood plasma of 5 dairy calves.

than those found during the subsequent colostral feeding period, which is in agreement with the observations of Green and Macaskill (10). Much of the early work on the lipid phosphorus levels in blood plasma involved the use of

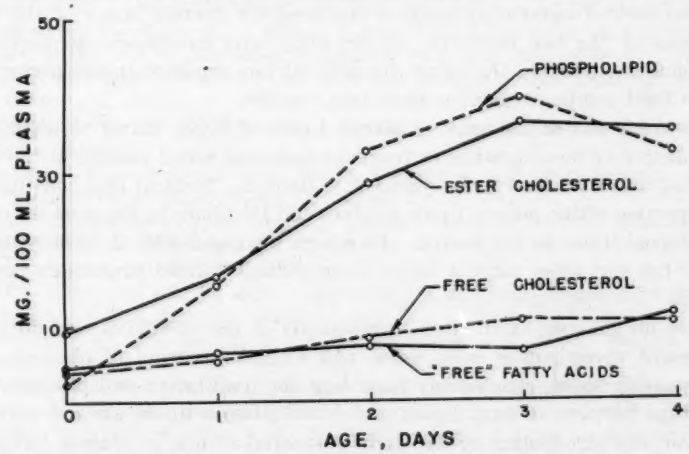

FIG. 2. Early post-natal changes, at daily intervals, in the mean phospholipid, cholesterol and "free" fatty acid values of the blood plasma of 5 dairy calves.

colorimetric or nephelometric measurements and the results included all of the phosphorus compounds that were soluble in alcohol-ether. In the present investigation, however, only those plasma phosphatides that were precipitated by acetone and $MgCl_2$ are reported as phospholipids. The possibility of the presence of other lipid-phosphorous components in calf blood plasma is recognized and will receive consideration in future reports.

In the present investigation both free and ester cholesterol were present in the blood plasma of the newborn calf. This is contrary to the observations of Shope (14) who found no ester cholesterol in the plasma of the calf at birth. Further observations of this investigator, however, indicated significant increases in ester cholesterol levels during the first few days after birth, a trend which is confirmed by the current study (fig. 2).

The mean percentages of ester cholesterol in the total cholesterol of blood plasma from calves at birth and at 1, 2, 3 and 4 days of age were 69, 73, 77, 78 and 77, respectively, indicating the attainment of a uniform percentage within 2 days after birth. It has been observed further (11) that no appreciable changes occur in calves restricted to milk diets during a subsequent 6-wk. period. In the mature bovine, however, somewhat lower values have been observed, 67 per cent by Boyd (7) and 69 per cent by Lo *et al.* (13).

The neutral fats followed trends similar to those of the phospholipids and ester cholesterol. Neutral fat values were obtained by calculation which was based on the assumption that the ester fatty acids were derived from saponification of cholesterol esters, phospholipids and triglycerides. When this method was employed by Boyd (7) in estimating plasma neutral fat for the mature bovine, the values were considerably higher than those obtained by other investigators (8, 12, 21) employing direct estimation by enzymatic hydrolysis. This apparent lack of agreement suggests the need for further study of the relative accuracies of the two methods. If the enzymatic hydrolysis technique is the more accurate, possibly the values for neutral fats reported in the present study include lipid components other than triglycerides.

The low levels of the various plasma lipids of dairy calves at birth suggest that transfer of these substances from the maternal blood plasma to the fetus is restricted and that fetal lipid synthesis is limited. Neutral fats constituted the major portion of the plasma lipids at birth and thus may be the most diffusible of the maternal lipids in the bovine. However, the possibility of fetal synthesis of neutral fat and other plasma lipids from diffusible lipid precursors cannot be ignored.

Since no analyses of the lipid components of the colostrum and milk fed in the present investigation were made and since the period of observation was comparatively short, conclusions regarding the qualitative and quantitative relationships between dietary lipids and blood plasma lipids are not warranted. Moreover, the significance of the early post-natal trends in plasma lipids is not apparent. The marked changes which were observed, however, emphasize the need for further investigation of these relationships over an extended age period.

SUMMARY

Changes in blood plasma lipids of Holstein and Brown Swiss calves were determined during the period from birth to 4 days of age. Increases in the values for total lipids, ester fatty acids, neutral fat, ester cholesterol and phospholipids were observed, whereas, no marked changes were apparent in free cholesterol and free fatty acid concentrations. A high correlation was found between the Allen fat values and the total lipids minus the sum of the phospholipids and free fatty acids.

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REFERENCES

(1) ALLEN, N. N. A Simple Volumetric Method for Determination of Fat in Blood Plasma. Proc. Soc. Exptl. Biol. Med., 31: 991–992. 1934.

(2) ALLEN, N. N. Blood Fat of Dairy Cattle. II. Factors Influencing the Fat Content of the Blood Plasma. Minn. Agr. Expt. Sta. Tech. Bull., 130: 17–52. 1938.

(3) BLOOR, W. R. The Fatty Acids of Blood Plasma. II. Distribution of the Unsaturated Acids. J. Biol. Chem., 59: 543–556. 1924.

(4) BLOOR, W. R. Plasma Lipoids in Anemia. J. Biol. Chem., 63: 1–15. 1925.

(5) BLOOR, W. R. The Oxidative Determination of Phospholipids (Lecithin and Cephalin) in Blood and Tissues. J. Biol. Chem., 82: 273–286. 1929.

(6) BOYD, E. M. The Oxidative Micro-estimation of Blood Lipids. Am. J. Clin. Path., 8: 77–90. 1938.

(7) BOYD, E. M. Species Variation in Normal Lipids Estimated by Oxidative Micromethods. J. Biol. Chem., 143: 131–132. 1942.

(8) CHANNON, H. S., AND COLLINSON, G. A. Blood Fat. II. The Acetone-ether Soluble Fraction. Biochem. J., 23: 1213–1221. 1929.

(9) CHUNG, A. C., SAARINEN, P., AND SHAW, J. C. A Comparison of the Allen Volumetric Blood Fat Procedure with an Extraction Procedure. J. Dairy Sci., 33: 918–921. 1950.

(10) GREEN, H. H., AND MACASKILL, E. H. Studies in Mineral Metabolism. VI. Comparison of the Blood of Cow and Calf in Respect to Mineral Constituents. J. Agr. Sci., 18: 384–390. 1928.

(11) JACOBSON, N. L., ZALETEL, J. H., AND ALLEN, R. S. Unpublished data. Ia. Agr. Expt. Sta. 1952.

(12) KELSEY, F. E., AND LONGENECKER, H. E. Distribution and Characterization of Beef Plasma Fatty Acids. J. Biol. Chem., 139: 727–739. 1941.

(13) LO, H. K., SAARINEN, P., AND SHAW, J. C. The Validity of the Allen Volumetric Procedure for the Determination of Blood Lipids of Cows on Different Feeding Regimes. J. Dairy Sci., 33: 922–924. 1950.

(14) SHOPE, R. E. The Effect of Age on the Total and Combined Cholesterol of the Blood Serum. J. Biol. Chem., 80: 141–148. 1928.

(15) STEWART, C. P., AND HENDRY, E. B. The Phospholipins of Blood. Biochem. J., 29: 1683–1689. 1935.

(16) THANNHAUSER, S. J. Serum Lipids and their Value in Diagnosis, Concluded. New Eng. J. Med., 237: 546–552. 1947.

(17) WILSON, W. R., AND HANSEN, A. E. Study of the Serum Lipids by a Micro-gravimetric Technique. J. Biol. Chem., 112: 457–468. 1935.

(18) WISE, G. H., PETERSEN, W. E., AND GULLICKSON, T. W. Inadequacy of a Whole Milk Ration for Dairy Calves as Manifested in Changes of Blood Composition and in Other Physiological Disorders. J. Dairy Sci., 32: 559–572. 1939.

(19) WITTCOFF, H. The Phosphatides. ACS Monograph Series, no. 112, pp. 152, 105. Reinhold Publishing Corporation, New York, N. Y. 1951.

(20) YASUDA, M., AND OHNO, K. The Simultaneous Micro-determination of Various Fractions of Lipids (Phospholipids, Cholesterol, and Fat) together with their Iodine Numbers. J. Biochem. (Japan), 30: 401–421. 1939.

(21) ZALETEL, J. H. Quantitative Assay and Iodine Values of the Fatty Acids of the Lipid Fractions of Cow's Blood Plasma. Unpublished M.S. Thesis. Iowa State College Library, Ames. 1950.

STUDIES OF HEATED MILK. IV. OBSERVATIONS ON BROWNING[1]

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When milk is subjected to prolonged heating, many complex chemical changes in the medium take place. One, of particular interest and significance, is that relating to color. Change in color occurs in many foodstuffs during heat-processing, storage or both. This phenomenon frequently is called "browning" or more specifically "non-enzymatic browning" to distinguish it from discolorations which are promoted by enzymes in raw fruits and vegetables. Among dairy products, browning is perhaps of most significance in evaporated milk, although sweetened condensed milk and dried milk products also are subject to the defect. Evaporated milk receives an appreciable degree of heat treatment during processing to render it sterile (117° C. for 15 min., on an average). This usually suffices to impart to the product a light tan color which is intensified during storage. Although much valuable information concerning browning of evaporated milk has been acquired, study of the problem has been hampered by lack of a convenient and precise method of measuring color. Measurement of browning in dairy products is complicated by the fact that the pigment is strongly associated with the proteins. Although one main school of thought, guided by the work of Kass and Palmer (8), believes the pigment to be physically adsorbed on the protein, it appears that nothing short of chemical degradation of the protein will free the pigment from it. In addition to arbitrary visual evaluation with or without the use of standards, determination of color in evaporated milk by measurement of reflectance has been proposed (11). Although the most valid approach to color measurement in opaque systems, its usefulness as a research tool is limited since it cannot be applied to translucent or transparent systems, a study of which is included in this paper. An alkaline extraction procedure for removing the brown pigment from dried milk and dried whey followed by photocolorimetric measurement of the extract has been employed by Doob *et al.* (2). Main objections to this method are the relatively inefficient extraction of color and limitation of its usefulness to dried products. Choi *et al.* (1) have developed a method which depends upon tryptic digestion of the milk protein to liberate color. Residual protein is removed by precipitation with trichloroacetic acid and color in the filtrate determined photometrically. This method appears equally applicable to fluid or dry milks and to systems of varying opacity. A preliminary evaluation of the method at this laboratory indicated it to be convenient and the results with it reproducible. Therefore, it was employed in the present study.

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The investigation consisted of two parts. The purpose of the first part was to determine to what extent browning occurs in present-day evaporated milk and to study the effect of storage at various temperatures on color development. In the second part, a study was made of the following factors which may be involved in the heat-induced browning of milk: Time and temperature, conditions of preheating, pH, the role of certain milk components, particularly casein, and the significance of certain compounds of importance in other browning systems.

Part I

EXPERIMENTAL

The samples of evaporated milk were obtained from commercial production in January and February, 1951. The freshly prepared products were furnished, together with information on their heat treatment, by 11 manufacturers. In order to defer any further development in color until after initial color determinations could be made, the samples were placed in refrigerated storage (4° C.) immediately on receipt.

All samples were packed in 14.5-oz. cans, with the exception of those from one manufacturer which were vacuum packed in a heavier 14-oz. lacquered can. Although there was considerable variation in forewarming treatment, all manufacturers used retort sterilization.

All color determinations were made according to the procedure of Choi *et al.* (1). A Klett-Summerson photoelectric colorimeter with no. 52 filter was used to measure optical density. By this method unheated milk samples gave uniform optical densities of 0.015 ± 0.005. Since these values were small and reasonably constant, all subsequent readings were made against distilled water as a matter of convenience. The evaporated milk samples were diluted with an equal volume of water before color analysis. Since the work of Choi *et al.* dealt mainly with dry milks, the optimum amount of trypsin (Nutritional Biochemicals Corp., 1–300 grade) to obtain maximum color liberation from the protein of an aged sample of evaporated milk was determined. Data essentially identical to those reported previously for dry milk (1) were obtained. Although nearly maximum color liberation was evident at a concentration of 150 mg. of trypsin per 25-ml. sample of milk, 2 ml. of a 10 per cent suspension of the enzyme were employed, rather than 1.5 ml. recommended by Choi *et al.*, to provide a margin of safety.

Although a complete critical evaluation of the tryptic digestion procedure was not a major purpose of the investigation, a number of significant observations were made in connection with it. The trypsin employed should be reasonably fresh. Satisfactory results were obtained with preparations stored for as long as 1 mo. at 4° C. However, a sample which had been held in the refrigerator for 6 mo. gave low values. The greatest problem with the method concerns occasional turbidity in the color filtrate. In nearly all instances this condition could be rectified by pouring approximately the first 5 ml. of filtrate back through the filter. The time interval between filtration and reading of color should be uniform, as there is a small but constant increase in optical density of the filtrate

on standing. Considerable data bearing on this matter were taken. The following are representative: A sample of evaporated milk in duplicate was digested with trypsin and residual protein removed by precipitation with trichloroacetic acid and filtration. Optical densities of the filtrate were determined immediately and after 8 and 16 min. standing. Values of 0.075, 0.079 and 0.083, respectively, were obtained. The results were identical for the duplicates. In order to minimize this time error throughout the study, color was measured as soon as 5 ml. of filtrate had accumulated. Such a procedure also was helpful in reducing the differences in speed of filtration between the evaporated milk samples. As pointed out by Choi *et al.* (1), it is difficult to ascertain how quantitative the method is, since there is no absolute basis of comparison. The appearance and quantity of residue left on the filter paper after tryptic digestion and acid precipitation of evaporated milk indicates that most of the color passes into the filtrate. The filter residue is largely fat, as evidenced by its melting to an oily mass on a steam bath. In an attempt to determine whether trypsin might affect

TABLE 1

Effect of trypsin and trichloroacetic acid in the tryptic digestion procedure on optical densities of heated[a] lactose-lysine systems[b]

pH of system[c]	Optical density		
	Trypsin and acid omitted	Acid added	Trypsin and acid added
6.18	0.011	0.010	0.009
6.96	0.019	0.020	0 020
7.67	0.048	0.056	0.054
8.46	0.231	0.266	0.270
9.50	0.600	0.820	0.830

[a] 120° C. for 20 min.
[b] Aqueous solutions containing 5.0% lactose and 0.3% lysine-HCl.
[c] Values before autoclaving, by adjustment with 10% NaOH.

pigments of the type present in evaporated milk, lactose-lysine systems having various pH values were browned in an autoclave and their colors evaluated with and without tryptic digestion. The results (table 1) indicated that trypsin has no effect on the pigments in these systems but trichloroacetic acid has a tendency to intensify the color slightly. However, color values obtained by the tryptic digestion procedure in this investigation always were consistent with the degree of color apparent to the eye.

Samples from all manufacturers were stored at room temperature (20 to 25° C.) and their color values determined at approximately monthly intervals for 1 yr. Samples from two manufacturers, one set vacuum- and one set air-packed, also were stored at 4 and 40° C. in order to study the effect of storage temperature on color development. Since the work of Tarassuk (20) has suggested that vacuum packing will improve the color of evaporated milk, the vacuum-packed product was included in the high- and low-temperature storage study.

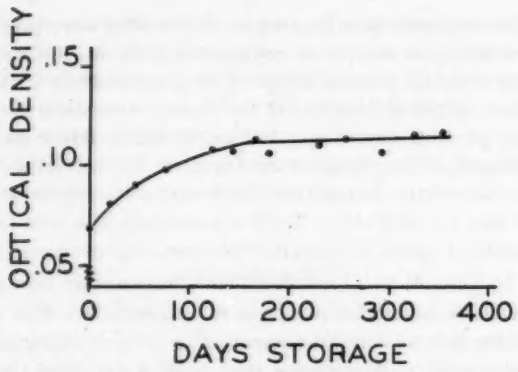

FIG. 1. The average color development in 11 commercial evaporated milks stored for 1 yr. at room temperature.

RESULTS AND DISCUSSION

The sterilizing treatment for the 11 commercial products ranged between 241 and 247° F. and 12- to 16-min. holding time. The average treatment was 243.2° F. for 14.3 min. There appeared to be little if any correlation between sterilization treatment and color. However, this might be expected in view of variations in milk supply, forewarming, cooling, use of stabilizing salts, etc. The trend in color development during storage of evaporated milk is shown in figure 1. The data are an average for the 11 products. Each of the products showed the same general trend although at different levels of color. The curve indicates rather rapid color development during the first 3 mo. of storage, after which the increase is quite gradual.

Data concerning the effect of various storage temperatures on color development in one air-packed and one vacuum-packed product are presented in figure 2. Greater color development occurs at higher storage temperatures. In fact,

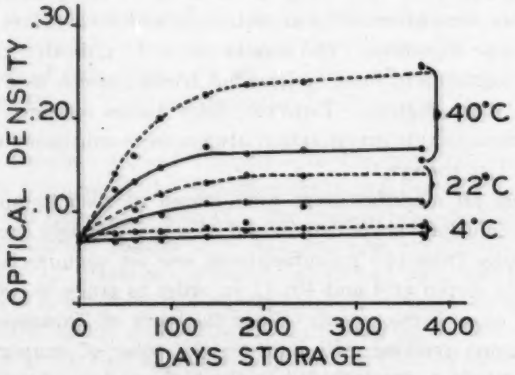

FIG. 2. The effect of storage temperature on color development during storage of an air-packed (————) and vacuum-packed (– – – – –) evaporated milk.

storage at 4° C. proved quite effective in preventing color formation during storage. The leveling-off process takes place at about the same time irrespective of the storage temperature. The vacuum-packed sample demonstrated greater color development at all temperatures than did the air-packed sample. This was true despite very similar initial color values for the two. Moreover, the vacuum-packed product developed more color on storage at room temperature than any other product included in the study. Tarassuk has found that the color of evaporated milk may be improved by reducing the oxygen available in the can before sterilizing (6). These limited data are not necessarily at variance with Tarassuk's findings, since his observations appear to have been confined to color evaluation immediately after sterilization and involved nitrogen rather than vacuum packing. However, they do suggest that vacuum packing of evaporated milk in a laquered can may be impractical from the standpoint of color development during storage.

In general, the results reported are in agreement with those of others (18, 22) on the effect of storage time and temperature on the color of evaporated milk. In addition, the data show that evaporated milk, as currently produced by a number of large concerns in this country, exhibits a discoloration defect at the time of manufacture and that this defect increases in intensity during storage.

Part II

EXPERIMENTAL

The following materials with sources as indicated were utilized in this part of the investigation: mixed-herd raw skimmilk, separated at 4° C. (College Creamery); calcium caseinate (Sheffield Farms Co., Inc.); β-lactose (Avoset Corp.); acetaldehyde (Distillation Products Corp.); methyl glyoxal (Union Carbide and Carbon Corp.); hydroxymethylfurfural, by synthesis (14); glycine, casein hydrolysates and trypsin 1–300 (all Nutritional Biochemicals Corp.).

The milk and simplified systems were heated in sealed 14.5-oz. evaporated milk cans. A standard sample volume of 390 ml. per can was used throughout the study. Heat treatment was carried out in an American Sterilizer Co. autoclave. Except in experiments where heat treatment was varied, uniform heating at 120° C. for 20 min. was employed. In all instances color was determined by the method of Choi *et al.* (1). Unless otherwise indicated, the colorimeter was zeroed with distilled water. pH determinations were made with a Beckman model M glass-electrode pH meter and adjustments in pH were accomplished with 10 per cent NaOH or HCl. The experiments reported have been conducted at least twice and in several instances many times; the results given are representative.

It is an acknowledged fact that the longer milk is heated, the browner it becomes (12, 20, 23). The effect of various time-temperature treatments on the color of autoclaved skimmilk was investigated. The results, which were very similar to those of Webb and Holm (23), showed that temperatures between 100 and 120° C. are quite critical with respect to color development. No noticeable change in color is evident in milk heated at 100° C. for 60 min.; however, above this temperature discoloration was progressively evident.

The fact that no significant amount of color was noted in the 100° C. series suggested that uncolored intermediates of browning may have been formed in some of these samples. To investigate this point, two sets of samples were autoclaved at 100° C.; pairs of samples were removed periodically throughout a 60-min. heating time. Color determinations were made on one of each pair and the other members of the pairs were autoclaved a second time at 120° C. for 20 min. Color values of the second group of samples were determined and compared with those of samples receiving one heat treatment. The results (table 2) indicate that small amounts of color precursors are formed, the color effect being most pronounced for the longer preheating times.

One factor which may be of major importance in the heat-induced browning of milk is pH (23). In order to study this variable, as well as the behavior of certain skimmilk fractions in browning, raw skimmilk, raw rennet whey and raw rennet whey from which the heat-coagulable proteins had been removed by heating to 80° C. at pH 5.5 were adjusted to various pH values, sealed in cans

TABLE 2

The effect of preheating at 100° C. for various time intervals on the amounts of color developed in the same samples of skimmilk when autoclaved at 120° C. for 20 min.

Preheating time	Optical density	
	After preheating	After autoclaving
(*min.*)		
0	0.016	0.105
7.5	0.016	0.107
15	0.016	0.105
30	0.017	0.125
45	0.016	0.138
60	0.020	0.144

and autoclaved. Color data for these samples are presented in figure 3. In this experiment only, pressure in the autoclave was not released immediately and rapidly through the safety valve at the end of the 20-min. holding period. Rather, the exhaust vent normally employed for open vessels was used, which required 20 min. for the autoclave to attain atmospheric pressure. As a consequence, the color values obtained in this experiment are not comparable with those from others reported herein. Nonetheless, these data amply demonstrate the importance of pH in the browning of milk by autoclaving. Normal milk having a pH of 6.6 to 6.7 falls at a critical point on the color curve. Relatively minor variations in pH toward greater alkalinity increased color significantly, while corresponding changes toward more acid values gave appreciable reduction in color. Figure 3 also reveals the importance of milk proteins, particularly casein, in the discoloration. Removal of casein by rennet coagulation greatly reduced the tendency of the medium to discolor. This reduction was augmented further by removal of the heat-coagulable proteins. Figure 4 demonstrates that the extent of discoloration in heated lactose-casein systems increases with increasing lactose content; in the absence of lactose, casein is resistant to heat-induced

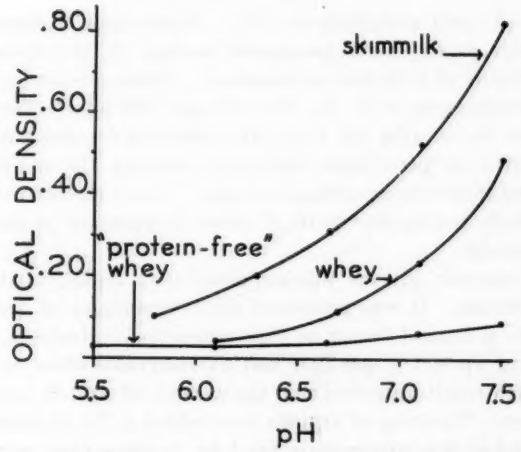

FIG. 3. The amounts of color developed by various milk fractions at different pH values as a result of autoclaving (120° C. for 20 min.).

browning. In this experiment various quantities of lactose were added to a 3 per cent calcium caseinate sol and the samples canned, autoclaved and cooled as previously described. After autoclaving at 124° C. for 20 min., 5 per cent lactose solutions without added casein exhibited no measurable color.

Several organic compounds appear to be involved directly or indirectly in the browning of certain media. Among these are hydroxymethylfurfural (7, 19),

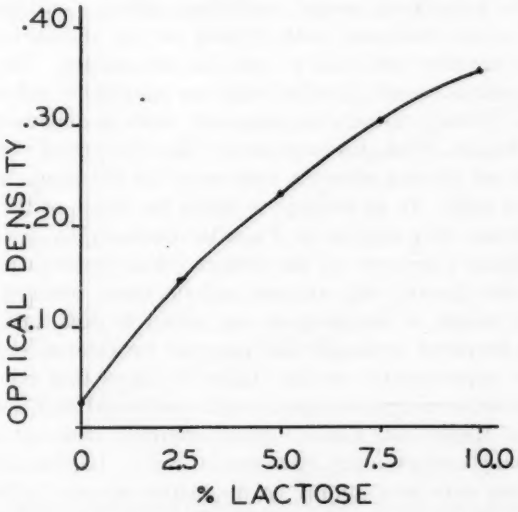

FIG. 4. The amounts of color developed by casein as a result of autoclaving (124° C. for 20 min.) in the presence of various quantities of lactose.

methyl glyoxal (3) and acetaldehyde (10). Since these compounds probably are formed in milk as a result of prolonged heating (9, 14), their possible contribution to browning of milk was investigated. These substances were added to skimmilk in concentrations of 0, 50, 100, 250 and 500 ppm. The samples were autoclaved, cooled in running tap water and observed for discoloration. Visual evaluation revealed no perceptible difference between the samples with compounds added and their corresponding controls. Under the conditions employed, these compounds do not appear to be of direct importance in the heat-induced browning of skimmilk.

In a similar manner, glycine was evaluated with regard to its capacity for augmenting browning. It was presumed that the amount of free amino acids in milk might be a critical factor in this connection. However, concentration of glycine ranging up to 1 g. per liter had no observable effect on discoloration. This unanticipated result suggested that the variable of protein hydrolysis should be studied further. Twelve g. of trypsin were added to 2 l. of skimmilk at 45° C. The milk was held at this temperature for 1 hr. to accomplish more or less complete digestion of its proteins. These conditions have been shown to be optimum for formation of non-protein nitrogen (1, and Part I this paper). The digested milk was divided into five 390-ml. samples, which were adjusted to various pH values and then sealed in cans. The maximum quantity of acid or base employed for pH adjustment was 5 ml. No correction for this small variation in sample volume was made. A similar group of samples was prepared from another 2 l. of the same skimmilk which had been handled identically to the first 2 l., except that no trypsin was added. The pH values of the undigested skimmilk before and after incubation were 6.67 and 6.53; of the digested skimmilk 6.58 and 6.15. The samples were autoclaved, cooled and their optical densities determined. This involved a second treatment with trypsin for the digested milk samples. Unheated control samples were used to zero the colorimeter. The use of these materials for standards seemed justified since no significant difference in color could be observed between autoclaved skimmilk, with and without inactivated enzyme added. Results from this experiment (figure 5) show that the tryptic-digested milk did not develop as much color over the pH range investigated as did the undigested milk. In an attempt to define the origin of the phenomenon, lactose-casein systems were studied in a similar manner. A calcium caseinate sol (3.2/l. containing 3 per cent of the protein) was prepared and divided in half. One-half was digested with trypsin and the other retained as a control. Five per cent by weight of lactose then was added to each half. Samples of varying pH were prepared, processed and observed for color as in the preceding experiment. The experimental results (figure 6) show that the difference in capacity to brown between tryptic digested and undigested milk mainly concerns the interaction of lactose and casein. This difference does not appear to be characteristic of only enzymatically hydrolyzed casein. Lactose-acid hydrolyzed casein systems gave data very similar to the lactose-enzyme hydrolyzed casein system.

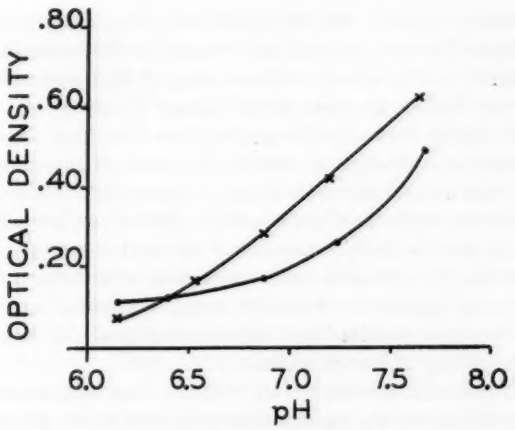

FIG. 5. The amounts of color developed by autoclaving (120° C. for 20 min.) skimmilk (X———X) and enzymatically digested skimmilk (● ——— ●) at various pH.

DISCUSSION

Evidence from this study has confirmed the importance of heat treatment, milk proteins, lactose and pH as variables in the heat-induced browning of skimmilk. Figure 3 shows that pH is important over a wide range of values. Some of the variation in the tendency of milks to brown on heating may arise from differences in pH. On the basis of these data, it also is logical to assume that the amount of alkaline stabilizing salt employed in the manufacture of evaporated milk may influence the amount of color in the product.

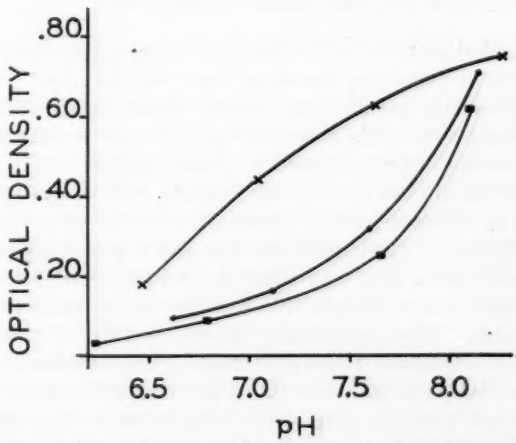

FIG. 6. The effect of autoclaving (120° C. for 20 min.) on the amounts of color developed in aqueous lactose-casein systems at various pH. Lactose + casein (■ ——— ■), lactose + enzymatically hydrolyzed casein (● ——— ●) and lactose + acid hydrolyzed casein (X———X).

The color values obtained with deproteinated whey (figure 3) suggest that inorganic phosphates are not of direct significance in the browning of autoclaved milk. This material, which should contain most of the inorganic phosphates of milk, produced very little color over the pH range 5.5 to 7.7. The slight amount of color obtained under these conditions may have resulted from the action of non-protein nitrogenous substances, rather than that of the phosphates. These results are somewhat at variance with those of others (21). The fact that small quantities of hydroxymethylfurfural, methyl glyoxal or acetaldehyde had no apparent effects on heat-induced browning of skimmilk lends support to the contention that browning is a general term which should be defined for the medium and the conditions of reaction. Although these compounds seem implicated in other browning systems, results here indicate that none of them are involved directly in the browning of heated milk.

Much of the fundamental research on browning has been conducted with simplified aqueous systems containing an amino acid, frequently glycine and a carbohydrate, usually glucose. Addition of glycine to milk in amounts up to 1.0 g.

TABLE 3

The amounts of 10% NaOH required to attain certain pH values in skimmilk before and after tryptic hydrolysis[a]

Skimmilk		Hydrolyzed skimmilk	
(pH)	(ml. of base)	(pH)	(ml. of base)
6.45	0.0	6.10	0.0
7.05	0.6	6.62	1.2
7.63	0.9	7.12	3.0
8.27	1.2	7.60	4.9
.....	.....	8.15	7.2

[a] Incubated at 45° C. for 1 hr. in the presence of 0.6% of trypsin.

per liter had no effect on color, and casein hydrolysates invariably were observed to be less effective in promoting browning than was the intact protein. A possible explanation of this phenomenon follows: Intact casein will produce more discoloration when heated with lactose than either acid or enzymatically hydrolyzed casein, as shown in figures 5 and 6. This suggests that casein has certain characteristics which are not directly attributable to its component amino acids. Among the unique characteristics of casein, its colloidal properties may account best for this difference. Evidence from this and a preceding paper (15) suggests that although casein may be present in a buffer at pH 6.6, such as it is in milk, entirely different conditions of pH may prevail at points on the surface of the protein particle. More specifically, in the vicinity of free amino groups distinctly alkaline conditions appear to exist. This difference in behavior between casein and amino acids seems to result from differences in particle size. The fact that casein exists in milk in the form of large dispersed particles appears to make it a relatively unresponsive buffer by which behavior the properties of its basic and acidic groups are preserved. On the other hand, amino acids are comparatively small molecules which dissolve readily and exhibit consider-

able buffer activity. Thus, the reactivity of their acidic and basic groups seems to be lowered. Data in table 3 reflect this difference in buffer activity as it occurs in skimmilk before and after tryptic digestion. The strong buffer activity of the digested milk is favorable to discoloration in that it counteracts a shift to more acid pH values during autoclaving; it is unfavorable in that it abolishes the distinctly basic character of certain functional groups in the protein. Therefore, the results presented in figures 5 and 6 are a net effect which probably would show even greater difference if buffer capacity were made constant. In further support of this contention, it has been observed that certain of the chemical reactions which occur when milk is heated are of a type which normally proceed under alkaline conditions. The more important compounds known to result when milk is heated are formic, acetic and lactic acids, acetol, maltol and furfuryl alcohol (5, 6, 9, 13, 16). These compounds also result from alkaline degradation of lactose or its components glucose and galactose (4, 13, 15). In general, sugars are more stable under acid conditions and, although the matter has been

TABLE 4

The amounts of certain compounds recovered from various simplified heated[a] systems and from heated[a] skimmilk

Systems[b] studied	Before heating	After heating	Furfuryl alcohol	Hydroxy-methyl furfural	Maltol
	(*pH*)	(*pH*)	(*g.*)	(*g.*)	(*g.*)
Lactose + Glycine (30 g.)	7.0	4.1	None	0.38	Trace
Lactose + Lysine (6 g.)	8.9	4.2	0.25	0.24	0.11
Lactose + $NaHCO_3$ (20g.)	8.3	4.8	0.87	Trace	None
Lactose + HCl	2.5	2.4	None	0.20	None
Condensed skimmilk	6.4	5.0	0.47	Trace	0.15
Condensed skimmilk + HCl	4.8	3.9	Trace	0.60	Trace

[a] 127° C. for 2.5 hr.

[b] 1-kg. quantities of 15% lactose solutions with the indicated materials added. 2-kg. quantities of skimmilk (30% total solids).

studied only partially, the indicated compounds do not appear to be formed readily from lactose under acid conditions. In heated milk, lactose has been shown to be the origin of all the mentioned compounds, and the presence of casein favorable or essential to their formation. Data in table 4[2] illustrate the differences in compound formation between lactose-amino acid systems and the lactose-casein system of milk. Bearing in mind that the amino group apparently is essential to maltol formation, it can be seen that the lactose-$NaHCO_3$ and lactose-lysine systems at pH values of 8.3 and 8.9, respectively, gave the same compounds on heating as the condensed skimmilk at pH 6.4. However, the glycine system at pH 7.0 gave results similar to the condensed skimmilk with pH of 4.8.

Thus, it seems clear that reactions involving lactose degradation which normally occur under alkaline conditions take place in milk at a measurable pH of 6.4 to 6.8 when the milk is heated. The particular chemical compounds formed, including those concerned with color, seem to be influenced by this phenomenon.

[2] A portion of these data and the methods relating thereto have been published (12).

SUMMARY

A method of rendering the brown pigment of evaporated milk soluble in milk serum by tryptic digestion of the proteins proved a satisfactory preliminary step to photoelectric measurement of color. Using this principle, the optical densities of 11 commercially produced evaporated milks were evaluated when fresh and at monthly intervals during storage for 1 yr. at room temperature. Discoloration was evident in all fresh samples. Color increased rather rapidly during the first 3 mo. of storage, after which time further color development was quite gradual. More color develops in samples held at higher storage temperature and storage at 4° C. will substantially inhibit color development. One vacuum-packed sample discolored more readily at all storage temperatures than did an air-packed sample of corresponding initial color. The investigation has demonstrated that "browning" is still a significant problem in the evaporated milk industry.

A number of factors involved in the heat-induced browning of skimmilk also were studied. These included time and temperature of heating, preheating, pH, the roles of certain milk components, particularly casein and the significance of certain compounds of importance in other browning systems. Casein and lactose were observed to be the principal reactants in production of the color. The temperature range 100 to 120° C. appeared critical in the browning of skimmilk. Heating for a period of 1 hr. at 100° C. produced no appreciable discoloration, whereas browning was readily evident in samples heated for 7.5 min. at 120° C. The tendency to discolor as a result of autoclaving was observed in decreasing order in skimmilk, rennet whey and "protein-free" rennet whey. The comparative resistance of the latter material to browning suggests that inorganic phosphates are not of direct importance in the heat-induced browning of milk. The region of pH 6.0 to 7.6 was found to be of critical importance in the browning of milk. A change of as little as 0.1 of a pH unit in this range produced a noticeable difference in color. Studies of the effect of preheating skimmilk on its tendency to brown on reheating suggests that browning in the case of milk is stepwise, at least in part. Although preheated skimmilks showed no difference in color, small differences proportional to the extent of preheating were observed following a second heat treatment. The additions of hydroxymethylfurfural, methyl glyoxal or acetaldehyde in quantities up to 0.5 g. per liter and glycine up to 1 g. per liter had no significant effect on heat-induced browning of skimmilk. Further study of the contribution of amino acids to browning in milk systems, established that milk subjected to tryptic digestion browns less readily than undigested milk and that either enzymatically or acid-digested casein browns less readily in the presence of lactose than does intact casein. In discussion of the phenomenon, it is suggested that intact casein may act in the manner of a stronger base than casein hydrolysates. Supporting evidence for this hypothesis is drawn from earlier work in which compounds isolated from heated milk to date also have been reported as products of the alkaline degradation of sugars.

ACKNOWLEDGMENTS

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REFERENCES

(1) Choi, R. P., Koncus, A. F., O'Malley, C. M., and Fairbanks, B. W. A Proposed Method for the Determination of Color of Dry Products of Milk. J. Dairy Sci., 32: 580–586. 1949.

(2) Doob, H., Willman, A., and Sharp, P. F. Influence of Moisture on Browning of Dried Whey and Skimmilk. Ind. Eng. Chem., 34: 1460–1468. 1942.

(3) Enders, C., and Marquardt, R. Methylglyoxal as Intermediary Product of Melanoidin and Caramel Formation. Naturwissenschaften, 29: 46–47. 1941.

(4) Evans, W. L., Edgar, R. H., and Hoff, G. P. The Mechanism of Carbohydrate Oxidation. IV. The Action of KOH on d-Glucose and d-Galactose. J. Am. Chem. Soc., 48: 2265–2277. 1926.

(5) Gould, I. A. Lactic Acid in Dairy Products III. The Effect of Heat on Total Acid and Lactic Acid Production and on Lactose Destruction. J. Dairy Sci., 28: 367–377. 1945.

(6) Gould, I. A. The Formation of Volatile Acids in Milk by High Temperature Treatment. J. Dairy Sci., 28: 379–386. 1945.

(7) Haas, V. A., Stadtman, E. R., Stadtman, F. H., and Mackinney, G. Deterioration of Dried Fruits. I. The Effect of Sugars and Furfurals. J. Am. Chem. Soc., 70: 3576–3579. 1948.

(8) Kass, J. P., and Palmer, L. S. Browning of Autoclaved Milk. Ind. Eng. Chem., 32: 1360–1366. 1940.

(9) Keeney, D. G., Patton, S., and Josephson, D. V. Studies of Heated Milk. II. Acetol and Related Compounds. J. Dairy Sci., 33: 526–530. 1950.

(10) Mohammad, A., Olcott, H. S., and Fraenkel-Conrat, H. The Reaction of Proteins with Acetaldehyde. Arch. Biochem. Biophys., 24: 270–280. 1949.

(11) Nelson, V. The Spectrophotometric Determination of the Color of Milk. J. Dairy Sci., 31: 409–414. 1948.

(12) Nelson, V. The Color of Evaporated Milk with Respect to Time and Temperature of Processing. J. Dairy Sci., 31: 415–420. 1948.

(13) Patton, S. The Isolation of Maltol from Heated Skimmilk. J. Dairy Sci., 33: 102–106. 1950.

(14) Patton, S. Studies of Heated Milk. I. Formation of 5-hydroxymethyl-2-furfural. J. Dairy Sci., 33: 324–328. 1950.

(15) Patton, S. Studies of Heated Milk. III. Mode of Formation of Certain Furan Compounds. J. Dairy Sci., 33: 904–910. 1950.

(16) Patton, S., and Josephson, D. V. The Isolation of Furfuryl Alcohol from Heated Skimmilk. J. Dairy Sci., 32: 222–227. 1949.

(17) Ramsey, R. J., Tracy, P. H., and Rushe, H. A. The Use of Corn Sugar in the Manufacture of Sweetened Condensed Milk. J. Dairy Sci., 16: 17–32. 1933.

(18) Simonson, H. D., and Tarassuk, N. P. Fluorescence and Associated Changes Produced upon Storage of Evaporated Milk. J. Dairy Sci., 35: 166–173. 1952.

(19) SINGH, B., DEAN, G. R., AND CANTOR, S. M. The Role of 5-(hydroxymethyl)-2-furfuraldehyde in the Discoloration of Sugar Solutions. J. Am. Chem. Soc., 70: 517–522. 1948.

(20) TARASSUK, N. Effect of Oxygen on the Color and Flavor of Heated Milk. Food Eng., 19: 781–783. 1947.

(21) WEBB, B. H. Color Development in Lactose Solutions during Heating with Special Reference to Evaporated Milk. J. Dairy Sci., 18: 81–96. 1935.

(22) WEBB, B. H., DEYSHER, E. F., AND POTTER, F. E. Effect of Storage Temperature on Properties of Evaporated Milk. J. Dairy Sci., 34: 1111–1118. 1951.

(23) WEBB, B. H., AND HOLM, G. E. Color of Evaporated Milk. J. Dairy Sci., 13: 25–39. 1930.

(24) WRIGHT, N. C. The Action of Rennet and of Heat on Milk. Biochem. J., 18: 245–251. 1924.

THE BACTERIOLOGY OF SWEET-CURD BRICK CHEESE[1, 2]

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The term "sweet-curd" describes a brick cheese which never becomes more acid than pH 5.1 and which has a moisture content of 44 per cent or slightly less. Control of pH and moisture is accomplished by washing the curd with water before dipping, thus removing much of the lactose. Conventional brick cheese made without water treatment often becomes too acid for proper curing when the moisture content approaches 44 per cent, the legal limit. The pH may drop to 4.8 or 4.9 and rise little, if at all, during ripening.

Foster *et al.* (6) described a curd-washing procedure with which they were able to control the acidity and moisture content of brick cheese within desired limits and produce cheese with good commercial characteristics. Buyens (3) also has used this method successfully and, in addition, has modified it to make it more acceptable in factory practice. The modifications involve largely a preliminary removal of most of the whey followed by application of a single volume of water, thus reducing the amount of whey to be separated and saving considerable labor. Buyens calls his procedure the "sweet-curd method." He states that at 6 to 8 wk. the sweet-curd cheese has a flavor resembling that of a combination of sweet Cheddar and mild, clean Limburger. It has an elastic body, it slices smoothly and its texture tends to be moderately close. At 6 wk. the moisture content of Buyens' cheese averaged between 43 and 44 per cent, the pH was 5.3 to 5.4 and the salt content was between 2.0 and 2.5 per cent.

Garey *et al.* (6, 8) studied the microbial flora of the interior of brick cheese made under various experimental conditions. They used pure cultures of *Streptococcus lactis* and *S. thermophilus*, individually and mixed, as starters for cheese made from raw and pasteurized milk by washed- and nonwashed-curd methods. They also studied the predominant organisms at different stages of manufacture and ripening (7). *S. lactis* was predominant throughout ripening of all cheese in which it was used as part or all of the starter. This organism was present in numbers of a few hundred million or more per gram, even after 6 wk. *S. bovis, S. faecalis, S. liquefaciens, Lactobacillus casei, L. brevis* and *L. lactis* were found in varying numbers in certain lots of cheese. From the results, however, it is impossible to draw any definite correlation between quality of the cheese and the presence or absence of any of these species.

The cheese studied by Garey *et al.* was made from milk of excellent bacterio-

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logical quality from a single herd. The work reported here was begun with the aim of determining the internal flora of brick cheese made from pasteurized mixed herd milk with a regular commercial starter by Buyens' sweet-curd method. It was known that good quality cheese could be made in this way and it was hoped that a study of the microbial flora might give some idea of the kinds of organisms that should be present and thus perhaps make it possible to insure their presence in any factory product. In addition, cheese made by conventional methods in commercial factories was selected for comparison.

EXPERIMENTAL METHODS

Unless otherwise indicated, the experimental cheese was made in the University of Wisconsin dairy from the regular mixed milk supply. The milk was pasteurized at 163° F. for 17 sec. and a regular *S. lactis*-type starter from a commercial source was used. This starter was employed regularly in the dairy for making other kinds of cheese. For convenience it is referred to hereafter as the U.W. starter. The sweet-curd cheese was made and cured by the method of Buyens (3). Precautions were taken at all stages of manufacture to avoid contaminating the milk and curd with extraneous organisms. Metal utensils were used and all objects to which the milk was exposed (including the cheesemaker's hands) were treated prior to exposure by immersion for 15 sec. to several minutes in a solution containing at least 100 ppm. of chlorine.

Most of the commercial cheese involved in the study was obtained from a single factory that reputedly produced a good quality product. The cheese was obtained soon after making and was cured in the University dairy. In addition, occasional samples of cheese from other factories were obtained from commercial sources.

The bacteriological and chemical methods of analysis described by Garey *et al.* (6, 7, 8) were used where applicable. Dilutions of the cheese were prepared in 0.9 per cent NaCl solution. Colony counts were made in carrot-liver (C-L) agar (8) shake tubes or plates incubated at 30° C. for 2 days. Foster *et al.* (6, 7) isolated organisms from the higher dilutions in this medium to obtain information on the predominant bacteria within the cheese. Obviously an organism could be present in reasonably high numbers and still not be detected by this procedure. Therefore, in this study selective media were used in an attempt to obtain information on groups of organisms that were not numerically predominant. Estimates of numbers of enterococci were made in the penicillin-azide agar of White and Sherman (13) and in the azide medium of Hajna and Perry (9) solidified with agar. Counts of non-lactic organisms were made in tryptone agar as described by Alford and Frazier (1). Coliform bacteria were detected by plating dilutions of cheese in violet red bile agar (2).

RESULTS

Total counts. Table 1 shows the C-L agar colony counts obtained from six representative lots of cheese made on different days. With one exception, all cheeses showed an increase in count to a maximum at 1 to 3 days, then a sharp

decrease at some time between 3 and 13 days. The maximum counts per gram usually were between 75×10^6 and 150×10^6. These dropped to a level of between a few hundred thousand and a few million per gram and then remained reasonably constant during further curing. Many other samples of cheese made by this method and sampled at 2 to 4 wk. invariably gave counts in the range of a few hundred thousand to 1 or 2 million per gram.

S. lactis-type organisms, presumably from the starter, were predominant at the time of the maximum count. Soon after the counts dropped, the isolates were micrococci or enterococci, but older samples yielded enterococci only. These had the characteristics of *S. faecalis*.

Lactose disappeared from most lots of cheese within 4 to 6 hr. after dipping. In all lots the pH dropped to 5.1 or 5.2 at about 2 to 3 days, then began to rise again until it finally reached a value of 5.25 to 5.3 at 4 wk.

The most unusual feature of the results in table 1 is the extremely low counts. Even the maxima are only about 1/10th to 1/20th as high as the counts obtained

TABLE 1

Carrot-liver agar colony counts from sweet-curd brick cheese made from pasteurized milk with the U. W. starter

Age of cheese	Colony counts/g. $\times 10^6$					
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
6 hr.	12	2.2	60	0.5	34	9
1 d.	75	22	110	10	150	120
2 "	62	.....	100	42	65	110
3 "	21	12	66	75	76	43
6 "	21	1.1	1.3	0.6	14	27
13 "	55	4.2	2	1.4	0.3	0.7
20 "	.....	2.1	1.9	0.4	0.03	0.2
27 "	.....	.....	1.8	0.8	0.05	0.4

by earlier workers (5, 6) from washed-curd cheese made with *S. lactis* in the starter. Since the methods of analysis used in the study reported here were identical with those employed before (5, 6, 7, 8) and since the rate of acid development in the cheese was normal, it was assumed that for some reason the majority of the organisms in the sweet-curd cheese failed to grow in the carrot-liver agar.

Table 2 shows C-L agar colony counts from three lots of brick cheese obtained from a near-by factory. The cheese had been made from pasteurized milk by a conventional (no wash) method. These counts are in the expected range and agree well with the results of the workers cited previously. Comparable counts have been obtained with ripened cheese from several other factories.

Reasons for the marked differences in the bacterial populations of the cheeses represented by tables 1 and 2 are not immediately apparent because the cheeses were made in different factories from different milk supplies with different starter cultures and by different methods. To control part of these variables, cheese was made from pasteurized milk in the University dairy with both the U.W. starter and the starter from the factory that supplied the cheese in table 2.

TABLE 2

Carrot-liver agar colony counts from 3 lots of commercial brick cheese[a]

Age of cheese	Colony counts/g. × 10^6		
	May 8, 1949[b]	May 10, 1949[b]	Nov. 12, 1950[b]
(*d.*)			
1	1,100		
2	2,000		2,800
3	800	1,400	
4	1,300	1.100	1,900
6	1,500		1,100
8	1,000	1,300	
10	1,100	1,200	200
15	900	1,100	
22	330	500	
30	340	430	
35	380	200	
44	250	220	

[a] Cheese made from pasteurized milk by a conventional (no-wash) method in a single commercial factory.
[b] Date of manufacture.

Each culture was used to make cheese by the sweet-curd method and by a conventional (no-wash) procedure. As a check on the method of counting the bacteria, direct microscopic clump counts were made on the cheese suspensions used for the C-L agar colony counts. In addition, estimates of numbers of viable organisms were made by preparing serial dilutions of the cheese in litmus milk, five tubes per dilution. From the results it was possible to estimate the "most probable numbers (M.P.N.) of organisms per gram by reference to the table in *Standard Methods for the Examination of Dairy Products* (2).

With each starter culture the bacteria counts were essentially the same in the cheese made by the sweet-curd method and in that made by the conventional procedure. Therefore, the differences shown by tables 1 and 2 cannot be explained on the basis of different making procedures. Results for the sweet-curd cheese are shown in table 3. Both starter organisms grew well in the fresh curd and

TABLE 3

Bacteria counts from sweet-curd brick cheese made from pasteurized milk with two different starter cultures

Age of cheese	Counts/g. × 10^6					
	U. W. starter			Factory starter[a]		
	DMCC[b]	MPN[c]	C–L[d]	DMCC[b]	MPN[c]	C–L[d]
4 hr.	870	1,100	120	500	220	70
2 d.	730	790	110	630	1,700	750
4 "	1,300	1,600	75	1,700	490	500
6 "	1,300	1,800	85	1,400	1,700	700
24 "	830	9	2	.....	5	2

[a] Starter from the factory that supplied the cheese in table 2.
[b] Direct microscopic clump count.
[c] Most probable numbers.
[d] Colony counts in carrot-liver agar plates incubated at 30° C. for 2 d.

reached the expected numbers of about 1×10^9 per gram, as shown by both the direct microscopic counts and the estimates of M.P.N. In the cheese made with the factory starter, the C-L agar colony counts were in reasonably good agreement with the counts made by the other methods, but in the cheese made with the U.W. starter the colony counts were much lower. They were, in fact, in the same range as the counts in table 1. Thus, there seems to be no doubt that the organisms in the U.W starter grew well in the cheese but did not readily form colonies in C-L agar.

Both starter organisms died off as the cheese aged, so that by 24 days only a few million viable bacteria remained per gram. The close agreement between the C-L agar counts and the M.P.N. estimates at this time suggest that the colony counts on cheese during the latter part of ripening are reliable (see table 1). The relatively high direct counts in the ripening cheese are normal because many of the organisms retain their ability to take the Gram Stain after they no longer are viable (8).

Several attempts have been made to obtain valid colony counts from young sweet-curd cheese made with the U.W. starter. Table 4 shows the results of two

TABLE 4

Bacteria counts from sweet-curd brick cheese made from pasteurized milk with the U. W. starter

Lot of cheese	Age of cheese	DMCC[a] $\times 10^9$	Colony counts/g. $\times 10^6$		
			Carrot-liver agar	Enriched carrot-liver agar[b]	BBL Eugonagar
	(*d.*)				
	1	1,300	80		160
1	2	1,600	50		75
	5	860	45	30	18
	11	460	0.35	0.15	0.5
	1	1,400	25		64
2	2	1,700	80		90
	5	880	75	95	45
	11	71	1.2	0.5	0.2

[a] Direct microscopic clump counts per g.

[b] Carrot-liver agar enriched with yeast extract, malt extract, Tween 80 and several other common medium ingredients.

trials with an enriched C-L agar and with BBL Eugonagar. Neither of these media was superior to the usual C-L formula. Other incubation times and temperatures were tried but none yielded counts higher than those obtained in the usual 2 days at 30° C.

Many trials have been made with the starter culture itself without finding the cause for the low counts in agar media. Of 45 colony counts made in various media from 15 different individual starter cultures 18 to 24 hr. of age, 33 were less than 20×10^6 per milliliter and only four were as high as the corresponding direct microscopic clump counts and M.P.N. estimates in litmus milk. The direct clump counts of all of the cultures were in the range of 300 to 600×10^6 per milliliter. Two of the high colony counts were obtained in plain C-L agar

and the other two were in this medium plus 1 per cent yeast extract. Other media tried were (a) BBL Eugonagar, (b) enriched C-L agar (see table 4), (c) a medium containing tomato juice, whey, tryptone, trypticase and yeast extract, as suggested by Tittsler (12) (d) the APT medium described by Evans and Niven (4) and (e) C-L agar plus 1 per cent skimmilk.

M.P.N. estimates in C-L broth were no higher than the corresponding C-L agar colony counts, although dilution cultures in litmus milk made at the same time indicated numbers comparable to the direct counts. Distilled water, Madison City water, Lake Mendota water and skimmilk were tried as diluents along with the usual 0.9 per cent NaCl solution. No one consistently yielded counts higher than the others.

Counts of enterococci in selective media. Since Foster *et al.* (7) demonstrated relatively large numbers of enterococci, particularly *S. faecalis*, in certain lots of brick cheese, it seemed desirable to determine whether these organisms also were numerous in the sweet-curd product. Two media selective for enterococci were tried. One was the "SF" azide medium of Hajna and Perry (9) modified by the addition of 1.1 per cent agar. The other was the penicillin-azide medium devised by White and Sherman (13) and used successfully by Kosikowsky and Dahlberg (11) to follow numbers of *S. faecalis* in Cheddar cheese made with this organism.

A preliminary experiment showed that stock cultures of *S. liquefaciens* and *S. faecalis* were markedly inhibited by the azide agar made from Hajna and Perry's formula (9). Even after 7 days, colony counts in this medium were only one-fourth to one-sixth as high as corresponding counts in C-L agar. *S. liquefaciens* showed no evidence of inhibition in the penicillin-azide medium but *S. faecalis* was inhibited completely. As expected, *S. lactis* and *L. casei* failed to grow in either selective medium. All four species grew normally in C-L agar, yielding numbers comparable to the direct microscopic clump counts.

The modified Hajna and Perry medium was tried with several lots of sweet-curd cheese and with two of the commercial lots shown in table 2. The counts of enterococci in the azide medium were much lower than they should have been as judged by the dilutions that yielded these organisms in C-L agar. For example, in one lot of sweet-curd cheese at 27 days the total count in C-L agar was 640,000 per gram and all of the cultures isolated from the highest dilution were *S. faecalis*. The corresponding azide agar count was only 800 per gram. Kosikowsky (10) recently reported similar inhibition of enterococci by the penicillin-azide medium. He has been able to obtain reasonably satisfactory results by reducing the penicillin concentration to 35 to 50 units per liter of agar.

Kosikowsky and Dahlberg (11) showed that certain lactobacilli could grow in the penicillin-azide medium. This has been true also in the work reported here with the agar medium made from Hajna and Perry's formula. Lactic rods comprised 20 to 80 per cent of the isolates from the azide medium cultures made from the commercial cheese represented by table 2 after the cheese was 3 days old or more.

Counts of nonlactic organisms in tryptone agar. The method of Alford and Frazier (1) was tried with some of the first lots of sweet-curd brick cheese to determine whether they contained appreciable numbers of nonlactic organisms. This method is based on the fact that the starter organisms grow poorly if at all in 1 per cent tryptone agar, hence other organisms present in smaller numbers can be detected.

The counts on tryptone agar varied considerably from one lot of cheese to another but they showed the same trends throughout the ripening period. At 3 to 6 hr. the counts were between 20,000 and 50,000 per gram, which is within the range reported by Alford and Frazier (1) for Cheddar cheese made from pasteurized milk. The numbers increased during the first 1 to 2 wk. to values of a few hundred thousand to a million per gram, then levelled off. Significantly this levelling off coincided with the marked drop in the C-L agar counts shown in table 1, and the numbers in the two media were practically identical during the later part of the ripening period. As might be expected from the similarity of the counts, the organisms isolated from the two media at this time were identical. Immediately after the marked drop in the C-L agar counts, the isolates from both media were either micrococci or enterococci. Later all were enterococci.

With some of the cheese samples there was considerable difficulty in making reliable colony counts in the tryptone agar because there was no clear-cut difference between organisms that grew well and those that did not. Colonies varying in size from 3 to 4 mm. down to a fraction of a millimeter in diameter were found, with all intermediate gradations.

Coliform organisms never were found on the tryptone agar plates of cheese made from pasteurized milk in the University dairy. In fact, these organisms never were detected in 0.01 g. of cheese of this type plated on violet red bile agar (2).

DISCUSSION

The results of this study show definitely that the bacterial flora of the sweet-curd cheese made from pasteurized milk in the University of Wisconsin dairy was not the same as that of similar cheese studied previously (5, 6, 7, 8) or that of cheese made in commercial factories. This difference was not great during the first few days after manufacture, but it became pronounced after 1 or 2 wk. of ripening. Because of the paucity of organisms within the sweet-curd product after the first few days, it is necessary to conclude that a large population of viable bacteria during ripening is not required for the production of a desirable cheese within the usual ripening period of 6 to 8 wk. In fact, one is tempted to believe that it may be more desirable *not* to have large numbers of living bacteria in brick cheese after the starter organisms die. A large population of miscellaneous bacteria actually may increase the chances of poor quality if the wrong ones are present in sufficient numbers.

The failure of organisms to grow in the cheese after the first few days is believed to be simply because none was present that could grow under the condi-

tions of curing. As previously stated, the milk was pasteurized at 163° F. for 17 sec. and the counts after pasteurization always were less than 2,000 per milliliter. In addition, the cheesemaker took special precautions to avoid introducing extraneous organisms during making.

The relatively low colony counts in the young cheese made with the U.W. starter must have been caused by the inability of most of the organisms in this culture to develop visible colonies in the agar media tried. The reasons for this still are not known, but it is necessary to accept the fact, in view of counts obtained by other methods, that these organisms grew well in the cheese. The regularity with which the counts dropped, even in C-L agar, seems to prove definitely that viable starter organisms did not persist in large numbers in the cheese for more than a few days.

Even though the C-L agar counts from the young cheese probably do not represent the true numbers of viable organisms, there is no apparent reason to question the validity of the counts made after the starter bacteria had died off at 1 to 2 wk. The predominant organisms after this time were types that grew well in all of the media tried. The close agreement between the M.P.N. estimates in litmus milk and the colony counts in agar (table 3) further support the validity of the colony counts from the ripening cheese.

SUMMARY

The bacterial flora in brick cheese made from pasteurized milk by a "sweet-curd" process has been described. *S. lactis*-type organisms from the starter grew rapidly and reached numbers of 1 to 2 billion per gram. Within 1 or 2 wk., however, the counts of viable organisms dropped to a few million or less per gram. The predominant organisms in the cheese during ripening were micrococci and/or enterococci, but, because of their relatively small numbers, it is believed that they had little to do with the ripening changes. Thus, it has been shown that brick cheese of excellent quality can be made in the usual period of 6 to 8 wk. without the action of large numbers of viable bacteria within the cheese during the ripening period.

The organisms in the commercial starter culture used in the University of Wisconsin dairy did not readily form colonies in carrot-liver agar or in any of the other solid media tried, although they grew normally in milk and in cheese. The starter organisms from commercial factories, however, consistently gave colony counts in carrot-liver agar comparable to the corresponding direct microscopic clump counts. The reasons for the deficiency of the agar media for the U.W. starter organisms are not known.

Two agar media containing sodium azide and supposedly selective for enterococci were tried for colony counts of these organisms, but both were inhibitory. As formulated, the efficacy of these media for quantitative estimation of enterococci is questioned.

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REFERENCES

(1) Alford, J. A., and Frazier, W. C. Occurrence of Micrococci in Cheddar Cheese Made from Raw and from Pasteurized Milk. J. Dairy Sci., 33: 107–114. 1950.

(2) American Public Health Association. *Standard Methods for the Examination of Dairy Products.* 9th ed. American Public Health Association, New York. 1948.

(3) Buyens, H. J. A Study of Methods of Controlling Acidity, Composition, and Ripening Properties of a Sweet-curd Type Cheese. Ph.D. thesis. Univ. of Wisconsin. 1951.

(4) Evans, J. B., and Niven, C. F., Jr. Nutrition of the Heterofermentative Lactobacilli that Cause Greening of Cured Meat Products. J. Bact., 62: 599–603. 1951.

(5) Foster, E. M. The Bacteriology of Brick Cheese during Ripening. Ph.D. thesis. Univ. of Wisconsin. 1940.

(6) Foster, E. M., Garey, J. C., and Frazier, W. C. The Bacteriology of Brick Cheese. II. Comparison of Washed-curd and Conventional Methods of Manufacture. J. Dairy Sci., 25: 313–321. 1942.

(7) Foster, E. M., Garey, J. C., and Frazier, W. C. The Bacteriology of Brick Cheese. III. The Bacteria Involved in Ripening. J. Dairy Sci., 25: 323–333. 1942.

(8) Garey, J. C., Foster, E. M., and Frazier, W. C. The Bacteriology of Brick Cheese. I. Growth and Activity of Starter Bacteria during Manufacture. J. Dairy Sci., 24: 1015–1025. 1941.

(9) Hajna, A. A., and Perry, C. A. Comparative Study of Presumptive and Confirmative Media for Bacteria of the Coliform Group and for Fecal Streptococci. Am. J. Pub. Health, 33: 550–556. 1943.

(10) Kosikowsky, F. V. Personal Communication. Department of Dairy Industry, Cornell Univ., Ithaca, N. Y. 1951.

(11) Kosikowsky, F. V., and Dahlberg, A. C. The Growth and Survival of *Streptococcus faecalis* in Pasteurized Milk American Cheddar Cheese. J. Dairy Sci., 31: 285–292. 1948.

(12) Tittsler, R. P. Personal Communication. Research Laboratories, Bureau of Dairy Industry, U.S.D.A., Washington, D. C. 1950.

(13) White, J. C., and Sherman, J. M. Occurrence of Enterococci in Milk. J. Bact., 48: 262. 1944.

THE EFFECTS OF ADDED CORN MEAL AND DRIED WHEY AND OF MOISTURE LEVEL ON THE PRESERVATION OF ALFALFA SILAGE[1, 2]

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The increase in the use of grass and legume silage has been accompanied by an increased interest in methods of preservation. Most of the reports indicate that moisture level and added preservative are two of the most critical factors in the preservation of silage of this type.

Stone *et al.* (12) showed that the fermentable sugar content of a grass crop is increased by wilting and concluded that moisture content did not influence preservation of grass silage as much as the presence of sufficient fermentable carbohydrate. Newlander *et al.* (9) found that soybean silage ensiled at 31 to 33 per cent dry matter was well preserved both with and without added molasses, while silage with 25 per cent dry matter had low quality when no molasses was added and fair quality when molasses was added. Their evaluation of silage quality was based on color, odor and palatability. A moisture content of 68 per cent or less is necessary for legume silage of the best quality, according to Woodward and Shepherd (15). Leakage and odor constituted the basis for their evaluation of quality.

Various carbohydrate materials have been used successfully to preserve grass silage. Both corn meal and molasses were proven satisfactory by King (3) and Stefaniak *et al.* (11). Allen *et al.* (1) and Nevens and Kuhlman (8) successfully preserved grass silage when dried whey was added. Molasses and ground barley each was found to be an excellent silage preservative by King (4, 5). Wilson and Webb (14) determined the water-soluble carbohydrate content of many common forages at various stages of growth and suggested that a relationship exists between moisture content of the plant, water-soluble carbohydrate content of the plant and preservation as silage. This experiment was designed to estimate the relative values of ground shelled corn and dried whey as silage preservatives when compared with a non-treated control. A comparison of wilted and unwilted alfalfa for silage was included in this experiment.

EXPERIMENTAL

Twelve miniature silos were used in this experiment. These silos, of concrete tile 6 ft. high and 2 ft. in diameter, were set on three slabs of concrete 5 ft. square

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and 6 in. thick. An additional 4 in. of concrete were added to hold the silos in place. A section of 1.25-in. pipe was set in the base of each silo as a drain.

The experiment was designed to compare two moisture levels (wilted and unwilted) with each of three preservative treatments. Alfalfa was cut in the one-tenth bloom stage. In both 1949 and 1950, half the alfalfa was chopped and ensiled as soon as possible after cutting. The remainder was allowed to wilt for approximately 1.5 hr. before being chopped and ensiled. Each of the six combinations was duplicated. Ground shelled corn added at the rate of 200 lb. per ton of green material was one treatment. Dried whey added at the rate of 30 lb. per ton of green material constituted another treatment. No preservative was added to the control group. The rate of application of corn meal was based on the work of Stefaniak *et al.* (11) and is a rate commonly used. Dried whey was added at the above rate to coincide with that used by Allen *et al.* (1) to supply about 1 lb. of lactose per 100 lb. of green material.

Preservative, when added, was weighed and mixed with the previously weighed alfalfa. Each alfalfa or alfalfa-preservative mixture was placed in a separate silo and thoroughly packed. Random samples were taken at the time each silo was filled. Each silo, when full, was covered with a double layer of tarpaper and a burlap bag containing 125 lb. of soil. The battery of twelve silos then was covered with a tarpaulin.

The silos were filled on August 25, 1949, and August 15, 1950, and emptied after periods of 6 mo. in 1949, and 6 wk. in 1950. Spoiled silage was separated from the unspoiled silage on the basis of appearance. Both were weighed and the percentage of spoilage was calculated. Sampling was accomplished by collecting random handfuls from the total quantity of unspoiled silage contained in each silo. These samples were frozen, stored and ground at a later date. The ground samples, weighing about 12 lb. each, were mixed thoroughly and an aliquot of about 1 lb. was frozen and stored for chemical analysis at a later time. Samples for carotene analyses were stored by the method described by Zscheile and Whitmore (16).

A.O.A.C. official methods (2) were employed in the determination of nitrogen, ash, crude fiber and ether extracts for both the materials as ensiled and the preserved silage. Dry matter was determined by drying weighed samples to constant weight in a vacuum oven at 70° C. Carotene was determined by slight modifications (6) of the methods of Zscheile and Whitmore (16) and Miller (7).

Total acidity, acetic acid, butyric acid, residual acidity, free amino acid and volatile base values of the silages were obtained by the method described by Watson and Ferguson (13). Statistical analysis of the data was accomplished by methods described by Snedecor (10).

In 1949 temperature and humidity conditions were such on the day of ensiling that the unwilted alfalfa contained only 60 to 65 per cent moisture by the time it was ensiled and the wilted alfalfa contained only 45 to 54 per cent moisture. Therefore, the alfalfa actually intended to be unwilted was of approximately the moisture content recommended by Woodward and Shepherd (15) for wilted silage. Rates of spoilage for 1949 are presented in table 1.

Applying the analysis of variance to the percentages of top spoilage, a significant difference between the moisture levels and among the three preservative treatments was found (6). Most of the variance among preservative treatments was due to low spoilage in the corn-treated silage as compared with spoilage of the untreated and whey treated silages. The interaction between moisture level and preservatives was significant; therefore, an investigation of the regressions of spoilage on moisture content seemed advisable.

The coefficient of regression (b in fig. 1) of spoilage on moisture content in the corn-treated silage varied considerably from the other two regression coefficients, indicating that moisture level affected spoilage less when corn meal was used as a preservative than when no preservative was applied or when whey was added. Analysis of variance among the coefficients of regression yielded an F value of 8.26 (six and two degrees of freedom) which was significant at the probability level of 0.05.

In 1950, the moisture levels were very uniform, with the silages ranging from 60.75 to 68.45 per cent moisture; therefore, no moisture level comparisons were made. Average spoilage for the three preservative treatments were corn 17.33

TABLE 1

Average percentages of top spoilage (1949)

Moisture level	Preservative			
	Untreated	Corn	Whey	Av.
Unwilted (60–65% moisture)	12.47	7.58	11.31	10.45
Wilted (45–54% moisture)	29.53	11.87	34.09	25.16
Av.	21.00	9.73	22.70	

per cent, whey 31.54 per cent and untreated 23.48 per cent. The day before the silos were emptied, the tarpaulin blew off three of the silos treated with whey and rain added to the weight of the spoiled material in those three, making an evaluation of the results of the 1950 trial difficult.

All of the silages in both years were green, had no unpleasant odors or flavors and were readily eaten by the dry cows and heifers to which the silages were fed.

Carotene content of the silages was determined in both 1949 and 1950 (see table 2). Carotene content of the material as ensiled was determined in 1949 but not in 1950. In 1949, the carotene content of the unwilted alfalfa was significantly higher than the wilted alfalfa at the time of ensiling, but, at the time the silos were emptied, the carotene content of the wilted silage was significantly higher. In 1950, also, the carotene content was significantly higher ($P = 0.01$) in the wilted silage than in the unwilted silage. As shown in table 2, however, differences are due almost entirely to differences between the whey-treated silages. Carotene values for both years were highly variable.

The materials ensiled and the silages in the 1949 trials were analyzed as previously outlined. Coefficients of preservation were calculated from these data and used in the statistical evaluation of treatment effects on the preservation of ether extract, crude fiber, nitrogen-free extract, crude protein and ash. Results of these analyses are presented on a percent of dry matter basis in table 3.

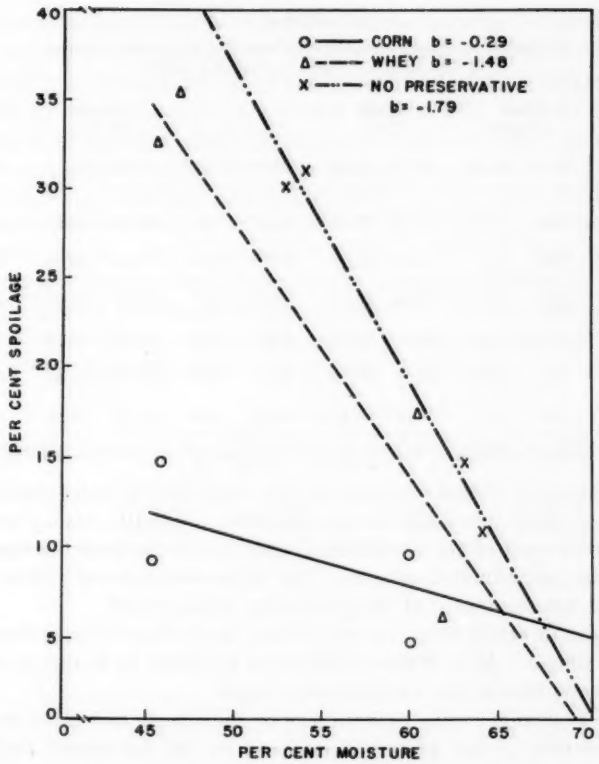

FIG. 1. The relationship between moisture content and spoilage of silages under the three experimental treatments.

Coefficients of preservation as presented in table 4 were derived by dividing the percentage in the unspoiled portion of the silage by that in the material ensiled.

The consistent increase in ether extract content from the time of ensiling to the time the silos were opened is most easily accounted for by the fact that the volatile acids produced by fermentation were extracted by ether in the analy-

TABLE 2

Carotene content expressed as γ per gram of dry matter

Treatment	1949				1950	
	Wilted		Unwilted		Wilted	Unwilted
	Material[a] ensiled	Silage	Material ensiled	Silage	Silage	Silage
Untreated	67.5	16.1	229.9	14.5	30.4	30.5
Corn meal	37.6	15.2	115.9	12.6	41.6	40.8
Dried whey	57.1	17.2	180.5	11.6	52.0	30.5

[a] Alfalfa or alfalfa-preservative mixture at the time of ensiling.

TABLE 3

Composition of materials ensiled and the unspoiled portion of the silages as per cent of dry matter

	Ether extract		Crude fiber		Ash		Crude Protein		Nitrogen-free extract	
	M E[a]	Silage	M E	Silage	M E	Silage	M E	Silage	M E	Silage
Unwilted Untreated	2.5	5.5	25.34	26.95	7.94	7.98	20.34	23.73	44.52	35.90
Unwilted corn meal	2.6	5.1	21.16	22.14	7.31	7.08	19.34	20.16	49.57	45.52
Unwilted dried whey	2.6	5.3	19.91	25.24	8.88	8.33	20.57	21.92	48.09	39.27
Wilted Untreated	2.8	4.8	23.87	24.75	7.93	7.82	21.36	22.73	44.03	39.92
Wilted corn meal	2.6	4.3	20.12	22.99	6.74	6.98	19.58	21.85	51.01	43.92
Wilted dried whey	2.0	4.7	23.29	25.17	7.89	7.85	21.08	21.55	45.75	40.81

[a] Material ensiled: alfalfa or alfalfa-preservative mixture at the time of ensiling.

sis. The analysis of variance applied to the coefficients of preservation of ether extract showed there was a significant difference ($P = 0.01$) among preservative treatments, with most of the significance being due to the large increase of ether extract in the whey-treated silages. The difference between wilted and unwilted silages was significant at the probability level of 0.05.

An increase in crude fiber content during the fermentation process was observed in all silages. Most of these differences appeared to be due to the greater increase in crude fiber in the whey-treated silages.

Ash content changed very little in any of the silages and there were no significant differences in ash preservation based on the calculated coefficients of preservation.

From the time of ensiling to the time of feeding, there was a slight increase of protein content in all treatments. There were no significant effects of treatment on protein preservation.

TABLE 4

Coefficients of preservation[a]

Treatment	Ether extract	Crude fiber	Ash	Crude protein	Nitrogen-free extract
Unwilted untreated	2.2	1.07	1.01	1.17	0.81
Unwilted corn meal	1.9	1.05	0.97	1.04	0.92
Unwilted dried whey	2.1	1.27	0.94	1.07	0.82
Wilted untreated	1.7	1.04	0.99	1.07	0.91
Wilted corn meal	1.7	1.14	1.04	1.11	0.86
Wilted dried whey	2.3	1.08	0.99	1.02	0.89

[a] Coefficient of preservation $= \frac{\text{composition of unspoiled silage}}{\text{composition of material ensiled}}$

Considerable reduction in nitrogen-free extract content could be accounted for by the action of bacteria on the fermentable carbohydrates. No significant effects of treatment on nitrogen-free extract preservation were observed.

The total (titratable) acidity, acetic acid, butyric acid, residual acidity (estimate of lactic acid) and volatile base values were variable. Moisture levels seemed to have the most consistent effects on these acid and base values. An attempt was made to correlate these values with spoilage, but due to the great variability and the relatively small number of observations, few significant correlations were found. No interpretation of the results of these analyses was made. Average values for each treatment are presented in table 5.

The pH values of the silages varied from 4.77 to 5.14 in 1949 and were not measured in 1950. There was no correlation between spoilage and pH and no significant difference in pH due to treatment of the silages.

TABLE 5

Average acid and base content of the silages expressed as 0.1N acid or acid equivalent per 100 g. of fresh silage

	Total acidity	Acetic acid	Butyric acid	Residual acidity	Volatile base
Unwilted untreated	701	193	2.22	293	100
Unwilted corn meal	769	155	7.79	437	88
Unwilted dried whey	712	154	8.24	369	86
Wilted untreated	674	93	0	458	71
Wilted corn meal	645	89	0	448	63
Wilted dried whey	673	84	0	485	67

SUMMARY

Ground shelled corn at the rate of 200 lb. per ton of green alfalfa at the time of ensiling was more effective in reducing top spoilage in miniature silos than dried whey added at the rate of 30 lb. per ton of green alfalfa. Dried whey, when used at the above rate, had no significant effect on the amount of top spoilage. Larger amounts of dried whey were not tested. Ground shelled corn reduced spoilage relatively more when applied to the wilted silage (45 to 54 per cent moisture) than when applied to the unwilted silage (60 to 65 per cent moisture).

There was less top spoilage in the unwilted silages than in the wilted silages. Neither moisture level nor application of preservative appreciably affected nutrient preservation in the unspoiled portion of the silage.

The wilted silages contained more carotene than the unwilted silages. The whey-treated silages, however, accounted for most of this difference.

Little variance was found in the pH of the silages and no correlation was found between pH and spoilage.

Total acidity, acetic acid, butyric acid, residual acidity and volatile base values are presented.

Although the rate of spoilage may not be the same in miniature silos as in larger silos, the relative differences between treatments probably would be similar.

REFERENCES

(1) Allen, L. A., Watson, S. J., and Ferguson, W. S. The Effect of the Addition of Various Materials and Bacterial Cultures to Grass Silage at the Time of Making on the Subsequent Bacterial and Chemical Changes. J. Agr. Sci., **27**: 294–308. 1937.

(2) Association of Official Agricultural Chemists. *Official and Tentative Methods of Analysis*, 6th ed. The Association, Washington, D. C., 1945.

(3) King, W. A. Comparison of Molasses-soybean Silage and Corn Meal-soybean Silage as Feeds for the Milking Cow. N. J. Agr. Expt. Sta. Bull. 713. 1944.

(4) King, W. A. Comparison of Molasses-grass Silage and Ground Barley Grass Silage as Feeds for the Milking Cow. N. J. Agr. Expt. Sta. Bull. 722. 1945.

(5) King, W. A. Comparison of Molasses-timothy Silage and Ground Barley-timothy Silage as Feeds for the Milking Cow. N. J. Agr. Expt. Sta. Bull. 723. 1945.

(6) Martin, T. G. The Effect of Method of Preservation on the Quality of Alfalfa Silage Stored in Miniature Silos. Unpublished M. S. Thesis, Iowa State College. 1951.

(7) Miller, E. S. A Rapid and Accurate Quantitative Method for the Determination of the Common Carotenoids; Analysis of Beta-Carotene and Leaf Xanthophyll in Thirteen Plant Tissues. J. Am. Chem. Soc., **57**: 347–349. 1935.

(8) Nevens, W. B., and Kuhlman, A. F. Alfalfa Silage. J. Dairy Sci., **19**: 611–617. 1936.

(9) Newlander, J. A., Ellenberger, H. B., Camburn, O. M., and Jones, C. H. The Conservation of Alfalfa, Timothy, and Soybean Nutrients as Silages and as Hays. Vt. Agr. Expt. Sta. Bull. 459. 1940.

(10) Snedecor, G. W. *Statistical Methods*, 4th ed. Iowa State College Press. Ames. 1946.

(11) Stefaniak, J. J., Rupel, I. W., Bohstedt, G., and Peterson, W. H. Methods of Preserving Glass Silage and Vitamin A Potency of Milk Produced Therefrom. J. Dairy Sci., **30**: 103–114. 1947.

(12) Stone, R. W., Bechdel, S. J., McAuliffe, H. D., Murdock, F. R., and Malzahn, R. C. The Fermentation of Alfalfa Silage. Penn. Agr. Expt. Sta. Bull. 444. 1943.

(13) Watson, S. J., and Ferguson, W. S. The Chemical Composition of Grass Silage. J. Agr. Sci., **27**: 1–42. 1937.

(14) Wilson, J. K., and Webb, H. J. Water-soluble Carbohydrates in Forage Crops and their Relation to the Production of Silage. J. Dairy Sci., **20**: 247–263. 1937.

(15) Woodward, T. E., and Shepherd, J. B. A Statistical Study of the Influence of Moisture and Acidity on the Palatability and Fermentation Losses of Ensiled Hay Crops. J. Dairy Sci., **25**: 517–523. 1942.

(16) Zscheile, F. P., and Whitmore, R. A. Determination of Carotene in Alfalfa: Studies on Methods for Meals and Fresh Leaves. Anal. Chem., **19**: 170–172. 1947.

AN ELECTROPHORETIC STUDY OF THE PROTEIN IN SKIMMILK[1]

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The physical and chemical properties of milk proteins have been studied by many investigators. In most cases the milk proteins were isolated by chemical methods and studied as separate entities, and there is some question as to whether these separated proteins exhibit the same characteristics as they do in their natural state in milk. Therefore, it was the purpose of this investigation to study the electrophoretic properties of the proteins of unfractionated bovine skimmilk over a range of pH's and ionic strengths and to compare the observed properties with those reported for the isolated components. In addition, a limited study of the effect of heat treatment on the unfractionated proteins of skimmilk was undertaken to demonstrate the possibilities of electrophoretic analyses as applied to unfractionated protein systems.

Mellander (8) examined unfractionated bovine skimmilk in a Tiselius electrophoresis apparatus at pH 6.9 and ionic strength 0.1 and reported the presence of four components, which he named α-casein, lactalbumin, β-casein, and γ-casein in the order of decreasing mobility. Mellander (8) also examined the casein fraction from bovine skimmilk and found three components, α-, β- and γ-casein. The casein fraction of bovine skimmilk was studied further by Krejci *et al.* (5), Krejci (4), and Warner (15). Warner described a chemical procedure for separating casein into its α- and β-components. Krejci *et al.* (5) and Warner (15) found electrophoretic evidence for the formation of a complex between α- and β-casein. Hipp *et al.* (2, 3) reported the isolation of γ-casein and have investigated its electrophoretic properties. The influence of rennet on α-casein has been studied by Nitschmann and Lehmann (9), who found that rennet treatment caused α-casein to split up into two electrophoretic components.

Electrophoretic studies of the whey proteins have been reported by Smith (12), Deutsch (1) and Stanley *et al.* (13, 14). The β-lactoglobulin of Palmer (10) was found to account for 63 to 70 per cent (by peak area) of the protein content of whey at pH 6.8, with the remaining 30 to 37 per cent of protein being distributed among five additional components (14). From this study and from the studies of Smith (12) and Deutsch (1), it is evident that Mellander's lactalbumin (8) corresponds to the β-lactoglobulin of Palmer. In this communication, the principal protein component of whey will be referred to as β-lactoglobulin, and the name lactalbumin will be reserved for a minor component of whey with a sedimentation constant $S_{20} = 1.9$ Svedbergs (11).

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PROCEDURE

Preparation of samples and buffer solutions. In the studies of untreated and unfractionated skimmilk proteins, fresh, mixed, unpasteurized cow's milk from the University herd was separated and cooled at once in ice water. Twenty-five ml. of skimmilk were diluted with 75 ml. of buffer and placed in a Visking dialysis membrane and dialyzed, with stirring, against approximately 300 ml. of buffer at 2° C. for 24 hr. The buffer then was discarded and the sample was dialyzed against 1,700 ml. of fresh buffer at 2° C. for 24 hr. At all pH levels above the isoelectric point, the samples were filtered through Whatman no. 1 filter paper to remove any slight flocculation that might have occurred during dialysis. When pH's below the isoelectric point were used, the milk samples were not diluted prior to dialysis, and insoluble materials were moved by centrifugation.

The buffer systems used were borate at pH 8.4, phosphate at pH 6.6, acetate at pH's 5.4 and 4.1 and lactate at pH 3.2. For each pH, three ionic strengths ($\Gamma/2$) were used, namely: 0.02, 0.10 and 0.50. In all solutions, the buffer ions were maintained at an ionic strength of 0.02, and for solutions with total ionic strengths of 0.10 and 0.50, NaCl was added in amounts equivalent to ionic strengths of 0.08 and 0.48 respectively.

In the studies of the effect of heat treatment on the electrophoretic properties of proteins in skimmilk, fresh skimmilk was heated to 65, 75 and 85° C., and held for 30 min. in a water bath. Heating was done in 1-l. flasks equipped with agitators and thermometers. At the end of the 30-min. period, the samples were cooled rapidly in ice water. The milk samples were prepared by diluting 15 ml. of milk with 85 ml. of phosphate buffer pH 6.6, $\Gamma/2 = 0.1$. Dialysis and electrophoretic analyses were conducted by the same procedures used for the unheated samples. Only one electrophoresis run was made for each temperature condition. A raw milk sample of the same milk was used as a control.

Electrophoresis measurements. The electrophoresis experiments were carried out in a Tiselius-Klett apparatus equipped with the schlieren scanning device of Longsworth (7). Cells of 92-mm. channel length were used. The electrophoresis cell was maintained at 0.5 ± 0.1° C. For the samples which received no heat treatment duplicate runs were made at all pH's and ionic strengths, except at pH 6.6, $\Gamma/2 = 0.10$, where four runs were made.

Mobilities were calculated from the velocities of the descending boundaries, employing the conductivities of the protein solutions measured at 0° C. The relative areas of the components in the schlieren photographs were determined by placing the negatives on finely ruled cross section paper and counting squares. Area measurements were repeated several times for each pattern and average values taken.

RESULTS AND DISCUSSION

Normal skimmilk. The schlieren scanning patterns of unfractionated and unheated bovine milk proteins are shown in figure 1, patterns (a) through (o) for pH's ranging from 8.4 to 3.2 and ionic strengths from 0.02 to 0.5. In these

figures, for a given pH and ionic strength, the patterns for both the descending and ascending boundaries are shown, with the pattern for the ascending boundaries on the left and the pattern for the descending boundaries on the right in each figure. The direction of migration is indicated by an arrow below each pattern and the initial boundary position is indicated by the position of the tail of the arrow. The mobilities of the descending boundaries and the relative areas of the schlieren peaks for ascending and descending boundaries are given in tables 1 and 2, respectively.

The principal components observed in the electrophoretic patterns of figure 1 were identified by comparison of their mobilities and relative areas with the

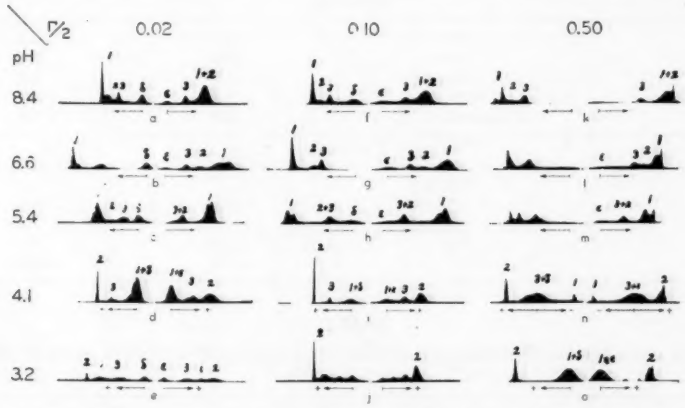

FIG. 1. Electrophoresis patterns of unheated skimmilk at various pH's and ionic strengths. Electrophoresis times and field strengths:

a. 90 min. at 8.11 volts/cm.	f. 200 min. at 4.43 volts/cm.	k. 760 min. at 2.57 volts/cm.
b. 125 min. at 8.83 volts/cm.	g. 180 min. at 6.38 volts/cm.	l. 714 min. at 2.48 volts/cm.
c. 90 min. at 8.35 volts/cm.	h. 280 min. at 4.91 volts/cm.	m. 990 min. at 2.50 volts/cm.
d. 132 min. at 5.49 volts/cm.	i. 198 min. at 5.52 volts/cm.	n. 825 min. at 2.53 volts/cm.
e. 60 min. at 7.73 volts/cm.	j. 115 min. at 5.75 volts/cm.	o. 726 min. at 2.52 volts/cm.

Components: α-casein (1), β-lactoglobulin (2), β-casein (3).

data of Mellander (8) for unfractionated milk at pH 6.9, $\Gamma/2 = 0.1$, and the data of Warner (15) on α- and β-casein for a range of pH's at $\Gamma/2 = 0.1$.

In the pattern of the descending boundaries for pH 6.6, $\Gamma/2 = 0.1$ (fig. 1-g) four principal components were observed. Listing them in the order of decreasing mobility, they were α-casein, β-lactoglobulin, β-casein and γ-casein plus ε-boundary.

At pH 8.4, $\Gamma/2 = 0.1$ (fig. 1-f, descending) only three major peaks were observed for the four possible components listed above. By a comparison of the relative areas of the peaks obtained at pH 6.6 and pH 8.4 (table 2) it was concluded that β-lactoglobulin migrated with α-casein at the higher pH. Similarly, at pH 5.4, $\Gamma/2 = 0.1$, (fig. 1-h, descending) only three major peaks were observed and on a comparison of peak areas with the peak areas of the pattern for

TABLE 1

Descending mobilities for the electrophoretic components of skimmilk at various levels of pH and ionic strength

Mobilities $\times 10^5$ (cm.^{2} sec.$^{-1}$ volt^{-1})
at 0° C.

pH	α-Casein	β-Lactoglobulin	β-Casein
	$\Gamma/2 = 0.02$		
8.4	5.7	5.7	2.8
6.6	6.4, 5.3	3.5	2.1
5.4	5.5, 4.6	1.4	1.4
4.1	0	+6.0	+3.6, +3.0
3.2	5.9	+7.5	+4.3
	$\Gamma/2 = 0.1$		
8.4	4.7	4.7	2.5, 1.2
6.6	4.8	3.0	2.0
5.4	4.1, 3.8	1.3	1.3
4.1	0	+3.9	+2.1
3.2	+4.2	+5.8	+2.0
	$\Gamma/2 = 0.5$		
8.4	2.6, 2.1	.....	.....
6.6	3.1, 3.0	2.3	1.8
5.4	2.8, 2.3	.....	1.4
4.1	1.5	+1.5	0
3.2	0	+3.0	+3.0

TABLE 2

Relative areas for the electrophoretic components of skimmilk at various levels of pH and ionic strength

pH	Ascending				Descending			
	α-Casein	β-Lacto-globulin	β-Casein	γ-Casein +δ	α-Casein	β-Lacto-globulin	β-Casein	γ-Casein +ε
				$\Gamma/2 = 0.02$				
8.4	20, 22	6	13, 4	35	←——73——→		22	5
6.6	44	9	21	26	34, 33	11	15	7
5.4	54	8	11, 8	19	36, 38	←—20—→		6
4.1	70*	19	6, 5	.....	51*	28	12, 9	.....
3.2	31	15	17, 16	21	42	16	22	20
				$\Gamma/2 = 0.1$				
8.4	40	11	17	32	←——72——→		22	6
6.6	51	18	24	7	62	11	19	8
5.4	29, 29	.....	25	17	35, 29	←—29—→		7
4.1	24*	62	14	.....	22*	52	26	.....
3.2	24	30	20	26	21	49	11	19
				$\Gamma/2 = 0.5$				
8.4	10, 38	21	31	.....	3, 16, 56	.....	17	8
6.6	47	.....	44	9	19, 41	13	18	9
5.4	21, 30	.....	37	12	23, 46	.....	25	6
4.1	8	26	66*	.....	8	31	61*	.....
3.2	73*	27	.....	.....	73*	27	.....	.....

* Includes area of δ- or ε-boundary.

pH 6.6 (see table 2) it was concluded that β-lactoglobulin migrated with β-casein at the lower pH.

At pH 4.1, $\Gamma/2 = 0.1$, (fig. 1-i, descending) three principal components were observed. From the mobility data of Warner (15) for α-casein and β-casein and of Li (6) for β-lactoglobulin, these components were identified as β-lactoglobulin, β-casein and α-casein, listed in the order of decreasing mobility.

At pH 3.2, $\Gamma/2 = 0.1$ (fig. 1-g, descending) four components were observed. The leading component was identified as β-lactoglobulin. It was not possible to identify positively the remaining components. The component of zero mobility was a new entity, and the two components between it and β-lactoglobulin were α- and β-casein, with some uncertainty as to their absolute identities.

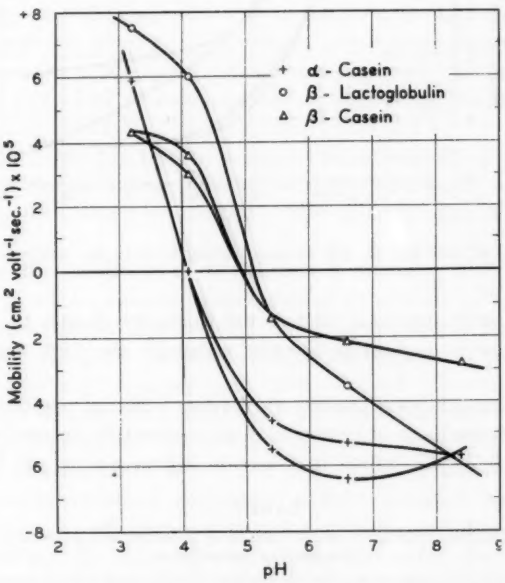

FIG. 2. Curves of mobility vs. pH at ionic strength = 0.02 for proteins in unfractionated skimmilk.

The influence of pH on the mobilities of the skimmilk proteins at $\Gamma/2, = 0.1$ is shown in figure 3. At $\Gamma/2 = 0.02$ and pH's from 8.4 to 3.2, the schlieren scanning patterns (figs. 2-a to e, descending), were quite similar to corresponding ones at $\Gamma/2 = 0.1$, and the identifications of components were readily made. The curves of mobilities vs. pH at $\Gamma/2 = 0.02$ are given in figure 2. From figure 2 and figures 1-b and 1-c, descending, it is evident that α-casein migrated as two components at pH's 6.6 and 5.4, which may be called α_1- and α_2-casein. The separation of α-casein into the α_1- and α_2-components also occurred at pH 5.4, $\Gamma/2 = 0.1$ (figs. 3 and 1-h, descending), but the difference in mobilities of the two components was small at this ionic strength. β-casein was found to divide into two components at pH 4.1, $\Gamma/2 = 0.02$ (fig. 3).

At $\Gamma/2 = 0.5$ and pH's from 8.4 to 5.4, the schlieren patterns for the skimmilk proteins showed some resemblance in number of components and relative peak areas to those for $\Gamma/2 = 0.1$ over the same range of pH's (figs. 1-k to 1-o). The principal differences in the patterns at $\Gamma/2 = 0.5$ and 0.1 occurred at pH's

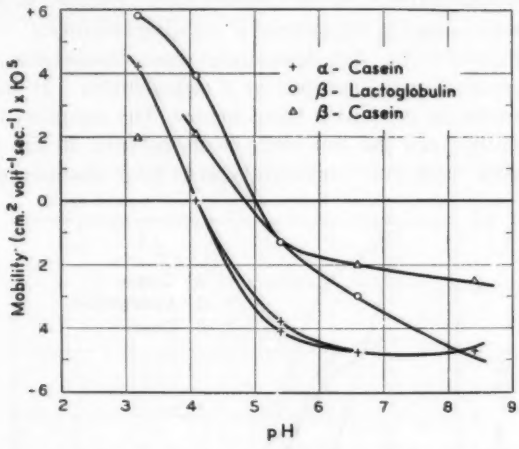

FIG. 3. Curves of mobility vs. pH at ionic strength = 0.10 for proteins in unfractionated skimmilk.

4.1 and 3.2, and were due to a shift of the isoelectric points to lower pH's and a general decrease in mobilities brought about by the high salt concentration (figs. 2 and 4).

From the electrophoresis studies of Warner (15) on whole casein and mixtures of α- and β-casein, it is known that an interaction occurs between α-casein and β-casein at $\Gamma/2 = 0.1$, which increases in degree as the pH is reduced from

TABLE 3

Influence of pH and ionic strength on interaction of α-casein and β-casein in skimmilk from descending boundaries

pH	Relative areas							
	$\Gamma/2 = 0.02$				$\Gamma/2 = 0.10$			
	α-Casein	β-Lacto-globulin	β-Casein	γ-Casein + ε	α-Casein	β-Lacto-globulin	β-Casein	γ-Casein + ε
8.4	62	11	22	5	61	11	22	6
6.6	67	11	15	7	62	11	19	8
5.4	74	11	9	6	64	11	18	7

8 to 5. This was shown by the increase in relative area of the α-casein peak and decrease in relative area of the β-casein peak which occurred as pH was decreased (15). Using the relative areas of the descending boundaries for $\Gamma/2 = 0.1$ given in table 3, and assigning a relative area of 11 per cent to β-lactoglobulin as de-

termined from the pattern at pH 6.6, it was possible to calculate the relative areas of α-casein and β-casein at pH's 8.4 and 5.4, where β-lactoglobulin migrated with one or the other of these components, by subtracting 11 relative units from the total relative area of the peak in which β-lactoglobulin occurred. The results of this analysis are given in table 3.

At $\Gamma/2 = 0.1$, it can be seen that only a slight increase in the relative area of the α-casein component and a correspondingly small decrease in the relative area of the β-casein component occurred with decreasing pH. Therefore, it was concluded that there is a very little interaction between the α- and β-casein components of unfractionated skimmilk at $\Gamma/2 = 0.1$. However, by applying the same analysis to the relative areas of unfractionated skimmilk proteins at $\Gamma/2 = 0.02$, a very marked increase in area of the α-casein component was observed and a correspondingly large decrease occurred in the area of the β-casein component with decreasing pH (see table 3). Therefore, it was concluded that there is an interaction between the α-casein and β-casein components of unfractionated skimmilk proteins at $\Gamma/2 = 0.02$, which increases in degree as the pH is decreased from 8.4 to 5.4.

At pH 8.4, $\Gamma/2 = 0.1$ (fig. 1-f), β-lactoglobulin is resolved as a separate peak in the patterns of the ascending boundaries and is within the α-casein peak in the pattern of the descending boundaries. This behavior also is seen at pH 8.4, $\Gamma/2 = 0.02$ (fig. 1-a).

In comparing the mobilities of the skimmilk proteins at $\Gamma/2 = 0.1$ (fig. 2) with the mobilities of the isolated components as reported by Warner (15) and Li (6), it was found that α- and β-casein and β-lactoglobulin in unfractionated skimmilk exhibited mobilities which were significantly lower than the mobilities of the chemically isolated components. Also, Warner (15) reported that the mobility of α-casein in unfractionated casein was lower than the mobility of purified α-casein. This comparison can now be extended to α-casein in unfractionated skimmilk, where this work has shown that the α-casein component has a lower mobility than the corresponding component in unfractionated casein.

These differences in mobilities between the proteins in unfractionated skimmilk and in the chemically isolated form indicate that either the procedures used in the isolation of the milk protein components produce changes in electrophoretic properties or there are interactions between the protein components in skimmilk which lower their mobilities.

The isoelectric points for unfractionated skimmilk proteins at $\Gamma/2 = 0.02$, 0.1, and 0.5 were obtained from the curves of figures 2, 3 and 4 and are listed in table 4.

Warner (15) reported the isoelectric points of isolated α- and β-casein as 4.1 and 4.5, respectively. This compares with the isoelectric points of 4.1 and 4.8 found in this investigation for the α- and β-casein components in unfractionated skimmilk. Pedersen (11) found the isoelectric point of pure β-lactoglobulin to be 5.2 at $\Gamma/2 = 0.02$. The isoelectric point for this component in unfractionated skimmilk was found to be 5.2.

At $\Gamma/2 = 0.02$, the isoelectric points of the skimmilk components (table 4)

were quite close to the values observed at $\Gamma/2 = 0.1$. However, at $\Gamma/2 = 0.5$, there was a shift in the isoelectric points of all components to lower pH values.

The best electrophoretic separation or resolution of the milk protein components of unfractionated skimmilk was obtained at pH 6.6 and $\Gamma/2 = 0.02$, with almost equal resolution at pH 6.6 and $\Gamma/2 = 0.1$. This may be seen by an examina-

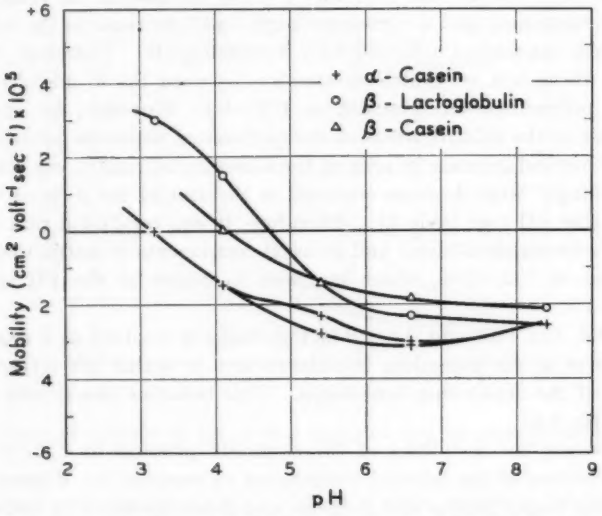

FIG. 4. Curves of mobility vs. pH at ionic strength = 0.50 for proteins in unfractionated skimmilk.

tion of the patterns (figs. 1-b and 1-g) and the mobility vs. pH curves (figs. 2 and 3). The commonly used veronal buffer (pH 8.6) does not permit good resolution of β-lactoglobulin from α-casein by electrophoresis, since β-lactoglobulin has nearly the same mobility as α-casein in this pH in the unfractionated skimmilk protein system.

TABLE 4

Isoelectric points of proteins in unfractionated skimmilk at various ionic sterngths

Ionic strength	Isoelectric points		
	0.02	0.1	0.5
Component			
α-casein	4.1	4.1	3.2
β-lactoglobulin	5.2	5.0	4.8
β-casein	4.9	4.8	4.5

Heat-treated milk. The electrophoresis patterns for the skimmilk samples which received 30 min. of heat treatment at 65, 75 and 85° C. are shown in figures 5-b, -c, -d. The patterns of the untreated control are shown in figure 5-a. In each figure, the descending boundaries are represented by the pattern on the right, and the ascending boundaries, by the pattern on the left. The relative

areas of the protein peaks in the descending patterns are given in table 4. As shown in figure 5 and table 5, heat treatment at 65° C. caused a reduction in the

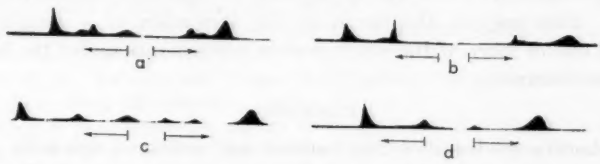

FIG. 5. Electrophoretic patterns of skimmilk at pH 6.6, $\Gamma/2 = 0.1$: (a) unheated, (b) heated at 65° C. for 30 minutes, (c) heated at 75° C. for 30 minutes, and (d) heated at 85° C. for 30 minutes.

concentration of β-lactoglobulin and the appearance of electrophoretically heterogenous material which was distributed between α-casein and β-casein peaks. From pattern c of fig. 5, it was evident that heating at 75° C. for 30 min. produced (a) a complete disappearance of β-lactoglobulin, and (b) considerable reduction in the concentration of β-casein and (c) the appearance of a new component of approximately zero mobility.

TABLE 5

Relative areas for the electrophoretic components of heat treated skimmilk at pH 6.6, $\Gamma/2 = 0.1$

Sample	Descending boundaries			
	α-Casein	β-Lacto-globulin	β-Casein	γ-Casein + ε-Boundary
Raw	64	13	23	0
65° C./30 min.	67	7	26	0
75° C./30 min.	76	0	11	13
85° C./30 min.	82	0	0	18*

* Mobility equals 0.56×10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$

TABLE 6

Descending mobilities for the electrophoretic components of heat treated skimmilk at pH 6.6, $\Gamma/2 = 0.1$

Sample	Mobilities $\times 10^5$ (cm.^{2} sec.$^{-1}$ volt^{-1}) at 0° C.		
	α-Casein	β-Lactoglobulin	β-Casein
Raw	4.6	2.87	1.98
65° C./30 min.	3.64	2.27	1.78
75° C./30 min.	3.66		1.3
85° C./30 min.	2.54		.56

From pattern d of figure 5, it was noted that β-casein and β-lactoglobulin were completely removed by heat treatment at 85° C. for 30 min., leaving only α-casein and a component of low mobility (table 5).

Mobilities of the descending boundaries are given in table 6. Here consistent and marked decreases in mobilities were observed for all components as the tem-

peratures of heat treatment were increased. It was noted that concurrent with the marked decrease in the mobilities of all components with increasing temperatures of heat treatment, there was an increase in total and relative concentration of α-casein. This may be the result of the formation of a complex between α-casein and one or more of the other protein components under the influence of elevated temperatures.

SUMMARY

Electrophoretic studies of unfractionated and unheated skimmilk proteins at ionic strengths of 0.02, 0.1 and 0.5 at pH's 8.4, 6.6, 5.4, 4.1 and 3.2 have shown that:

(a) The electrophoretic mobilities of the proteins in unfractionated skimmilk are appreciably lower than the mobilities of the corresponding isolated proteins.

(b) The isoelectric points of the proteins in unfractionated skimmilk are approximately the same as the isoelectric points of the corresponding isolated proteins reported by other workers.

(c) The best electrophoretic resolution of the proteins in skimmilk occurs at pH 6.6 and ionic strengths of 0.02 and 0.1.

(d) α-Casein migrates as two distinct components, α_1- and α_2-casein, over a range of pH's above 4.2 at ionic strengths of 0.02 and 0.10.

(e) An interaction occurs between the α- and β-casein components of skimmilk which increases in degrees as pH is lowered from 8.4 to 5.4 at ionic strength 0.02.

Heat treatments of skimmilk for 30 min. at 65, 75 and 85° C. were found to cause the following changes in the electrophoretic properties of the protein components as revealed by electrophoresis measurements at pH 6.6, $\Gamma/2 = 0.1$: (a) The mobilities of α-casein, β-casein and β-lactoglobulin are lowered by heating, the effect being greater at the higher temperatures; (b) The concentrations of β-lactoglobulin and β-casein are decreased and the concentration of protein migrating with α-casein is increased by heating skimmilk under the conditions given.

REFERENCES

(1) Deutsch, H. F. A Study of Whey Proteins from the Milk of Various Animals. J. Biol. Chem., **169**: 437–488. 1947.

(2) Hipps, N. J., Groves, M. L., Custer, J. H., and McMeekin, T. L. Separation of γ-Casein. J. Am. Chem. Soc., **72**: 4928–4931. 1950.

(3) Hipp, N. J., Groves, M. L., Custer, J. H., and McMeekin, T. L. Separation of α-, β- and γ-Casein. J. Dairy Sci., **35**: 272–281. 1952.

(4) Krejci, L. E. Electrophoretic Evidence for Complex Formation in Casein, A Correction. J. Franklin Inst., **234**: 197–201. 1942.

(5) Krejci, L. E., Jennings, R. K., and Smith, L. D. Electrophoretic Evidence for Complex Formation in Casein. J. Franklin Inst., **232**: 592–596. 1941.

(6) Li, C. H. Electrophoretic Inhomogeneity of Crystalline Beta-lactoglobulin. J. Am. Chem. Soc., **68**: 2746–2747. 1946.

(7) Longsworth, L. G. Optical Methods in Electrophoresis. Ind. Eng. Chem., Anal. Ed., **18**: 219–229. 1946.

(8) Mellander, O. Electrophoretische Untersuchung von Casein. Biochem. Z., **300**: 240–245. 1939.

(9) NITSCHMANN, H., AND LEHMANN, W. Electrophoretische Differenzierung Von Saure— und Labcasein. Experimentia, 3: 153–155. 1947.

(10) PALMER, A. H. The Preparation of Crystalline Globulin from the Albumin Fraction of Cow's Milk. J. Biol. Chem., 104: 359–372. 1934.

(11) PEDERSEN, K. O. Ultracentrifugal and Electrophoretic Studies of the Milk Proteins. Biochem. J., 30: 948–960. 1936.

(12) SMITH, E. L. Isolation and Properties of Immune Lactoglobulins from Bovine Whey. J. Biol. Chem., 165: 665–676. 1946.

(13) STANLEY, W. G., ANDREWS, A. C., AND WHITNAH, C. H. Electrophoresis of Milk Proteins. I. Some Comparisons of Salt-acid and Salt-lyophilized Whey Fractions. J. Dairy Sci., 33: 275–287. 1950.

(14) STANLEY, W. G., WHITNAH, C. H., AND ANDREWS, A. C. Electrophoresis of Milk Proteins. II. Some Effects of Methods of Preparation on the Electrophoretic Patterns of Whey Proteins. J. Dairy Sci., 34: 265–272. 1951.

(15) WARNER, R. C. Separation of α- and β-Casein. J. Am. Chem. Soc., 66: 1725–1731. 1944.

THE EFFECT OF AUREOMYCIN FEEDING ON CHANGES IN WEIGHT AND IN BODY MEASUREMENTS OF DAIRY CALVES[1, 2]

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The growth-stimulating effect on young dairy calves of orally administered aureomycin has been demonstrated by numerous investigators (1, 9, 10, 12, 14), whereas with older dairy cattle and with other ruminants the data are neither so extensive nor so conclusive. Although adverse effects were observed when aureomycin was fed to steers (2) and to lambs (4, 5, 6), favorable responses have been reported when this antibiotic was administered to ruminating dairy calves (8, 11) and no adverse effects were noted when it was fed to dairy cows (7). Inasmuch as further study of the effects of antibiotics on older dairy animals seemed desirable, the present investigation was undertaken to determine the responses of ruminating dairy calves (a) to continued aureomycin supplementation over a comparatively long period of time, (b) to introduction of aureomycin into the diet and (c) to removal of aureomycin from the diet.

EXPERIMENTAL

Thirty-two dairy calves which had been employed in an earlier study (10) were divided at 116 days of age into four comparable groups with breed and sex distribution as shown in table 1. Half of the calves previously had received aureomycin and were divided in the present investigation so that one group (AA) continued to receive the antibiotic, whereas supplementation was discontinued in the other group (AC). The calves which had not been fed aureomycin previously likewise were divided, one group (CA) receiving the antibiotic during the present study and the other (CC) receiving none. Each calf had received hay, a concentrate mixture and either whole milk or reconstituted skimmilk previously and allotment to groups was such that a comparable number of animals in each group had been fed each of the two types of milk.

During the present investigation (age period, 117 to 200 days), each calf was placed in an individual pen and was fed a concentrate mixture,[4] limited to a maximum of 4 lb. per calf daily, and alfalfa hay, salt and water *ad libitum*. The aureomycin hydrochloride fed to groups AA and CA was in a feed supplement (Aurofac A[5]) and was mixed with the concentrate.

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[2] Supported in part through funds provided by Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y., and by Western Condensing Co., Appleton, Wis.

[3] Present address: Ottawa Dairy Div., The Borden Co. Ltd., Ottawa, Can.

[4] Composed of 27 per cent ground corn, 30 per cent crushed oats, 13 per cent wheat bran, 13 per cent soybean oil meal, 7 per cent linseed oil meal, 7 per cent dried beet pulp, 2 per cent steamed bone meal and 1 per cent salt.

[5] This supplement, containing not less than 1.8 g. aureomycin hydrochloride per pound, was supplied by Lederle Lab. Div., American Cyanamid Co., Pearl River, N. Y.

Criteria for evaluation of the responses of the calves to the various treaments were health; changes in body weight recorded at weekly intervals; changes in height at withers, chest circumference, chest depth and barrel circumference recorded at 4-wk. intervals; and daily consumption of concentrates, hay and water.

TABLE 1

Breed and sex distribution of calves in the various dietary groups

Breed		H		J		G		A	
Sex		M	F	M	F	M	F	M	F
Previous treatment[a]	Experimental treatment[b]	(No. of animals)							
A	A	3	1	1	1	1			1
A	C	3	1	1	1		1	1	
C	A	3	1	1	1	1			1
C	C	3	1	1	1		1	1	

[a] During the age period from 4–116 d. A = 80 mg. crystalline aureomycin hydrochloride/calf daily; C = no aureomycin supplementation.

[b] During the age period from 117–200 d. A = 80 mg. aureomycin hydrochloride (in Aurofac A)/calf daily; C = no aureomycin supplementation.

RESULTS

The group mean weekly weights during the 12-wk. experimental period are presented in figure 1. The calves fed aureomycin previous to and during the experimental period (group AA) weighed more initially (116 days of age) than

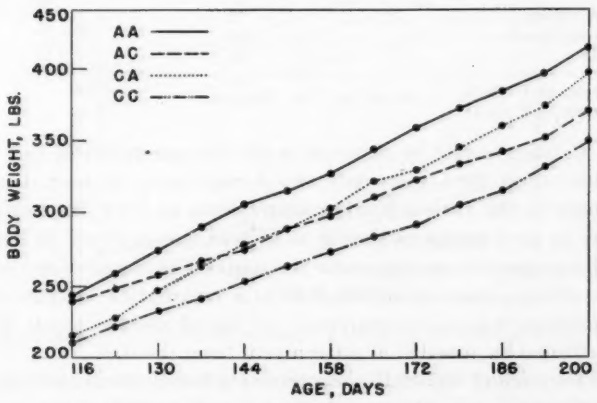

FIG. 1. Mean changes in body weights of calves in the various dietary groups.

the calves which received no antibiotic (group CC). Moreover, the weight gains during the experimental period were greater for calves in the former group than for those in the latter. Removal of aureomycin from the ration (group AC) resulted in an abrupt diminution in rate of growth, whereas introduction of aureomycin into the diet of calves not previously fed the antibiotic (group CA) resulted in an early increase in growth rate.

An analysis of variance (13) was employed to evaluate statistically the body weights (table 2). Only the data for the Holsteins and Jerseys were used in this analysis, since the sex distribution was not uniform for the other breeds (table 1). The weight changes of calves fed aureomycin during the present experiment (groups AA and CA) were significantly greater ($P = 0.01$) than those of calves that did not receive the antibiotic (groups AC and CC). Moreover, calves not fed aureomycin previously (groups CA and CC) gained significantly more ($P = 0.05$) than those fed aureomycin prior to the present investigation. There was no significant interaction, however, between previous treatment and experimental treatment. Conversely, the interaction between breed and experimental treatment was significant at $P = 0.05$, indicating a differential response of the two breeds to aureomycin feeding. The mean daily weight gains of Jersey calves in the aureomycin and in the non-supplemented groups were 2.1 and 1.1 lb., respectively, whereas those for the comparable Holsteins were 2.2

TABLE 2

Analysis of variance of total body weight changes during the 12-week experimental period

Source of variation	Degrees of freedom	Mean square
Treatment	3	4,062.0**
Previous treatment	1	1,365.0*
Experimental treatment	1	10,795.0**
PT X ET	1	26.1
Breeds	1	10,121.0**
B X T	3	1,585.3*
Sex within breeds	2	918.5
S X T within breeds	6	283.6
Individuals	8	232.2

* Significant at $P = 0.05$.
** Significant at $P = 0.01$.

and 1.8 lb. Caution should be observed in the interpretation of this differential breed response, since there were only two Jersey calves in each dietary group.

The changes in the various body measurements at 4-wk. intervals, shown in figure 2, were in most instances similar to the changes observed in body weights. Initiation of aureomycin supplementation resulted in an acceleration, and discontinuation of supplementation resulted in a retardation, of rate of increase. The only exception was in circumference of barrel measurements in which no change was effected by removal of aureomycin from the diet.

The data for each of the body measurements for Holsteins and Jerseys were analyzed by the same procedure as that employed in table 2 for body weights. Although the trends in height at withers and in chest depth were similar to those observed in body weights, the differences were not significant statistically at $P = 0.05$. The changes in circumference of chest and in circumference of barrel, however, were significantly greater ($P = 0.01$) for calves fed aureomycin (groups AA and CA) than for those which received no antibiotic (groups AC and CC). It also was observed that the animals that had not received aureomycin previously (groups CA and CC) exhibited significantly ($P = 0.01$) greater positive

changes than those that had been fed the antibiotic (groups AA and AC). There was no apparent interaction between previous treatment and present treatment, whereas a significant ($P = 0.05$) interaction between breed and treatment was observed for both measurements, the Jerseys exhibiting the greater response to aureomycin supplementation.

The mean feed consumption, in pounds, for calves in groups AA, AC, CA and CC, respectively, were: hay, 616, 516, 567 and 474; concentrates, 336, 336, 336 and 330; and water, 3,009, 2,757, 2,940 and 2,778. Since the concentrates were lim-

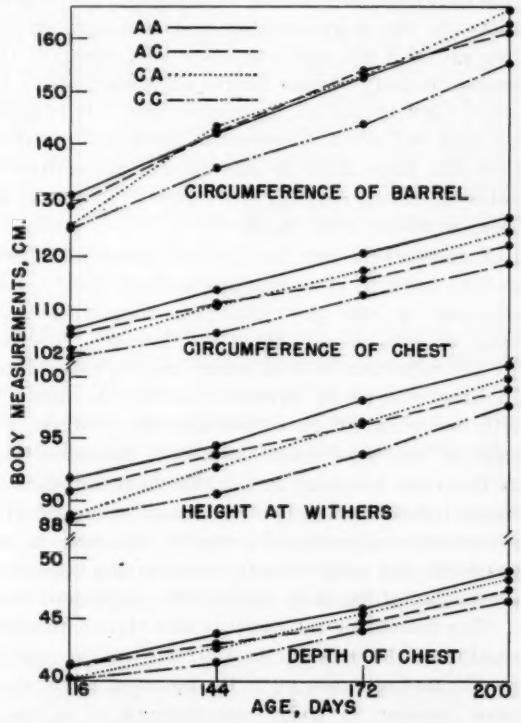

FIG. 2. Mean changes in body measurements of calves in the various dietary groups.

ited to 4 lb. per calf daily, the consumption in all groups was approximately the same. Hay and water consumptions, however, were greater for calves fed aureomycin. The hay boxes were designed to minimize wastage. Nevertheless, there was some loss and thus the actual consumption was slightly less than the values indicated. There was no reason to believe, however, that there were any appreciable differences among the various groups in the amount of hay that was wasted.

In general, the physical condition of calves in all groups was highly satisfactory. Elevated body temperatures were observed in a few individuals but no inter-group differences in rate of occurrence were apparent. All cases re-

sponded to sulfonamide therapy. One calf in group CC exhibited a depressed appetite and a sub-normal blood plasma vitamin A level (both of which were alleviated soon after administration of massive doses of vitamin A) and another in the same group scoured during a brief period. Two mild cases of bloat occurred in two calves in group CA. Condition of haircoat was markedly superior among calves fed aureomycin.

DISCUSSION

The accelerated growth rate previously observed (10) among calves fed aureomycin from 4 to 116 days continued during the period of the present investigation. The difference between the supplemented and non-supplemented groups increased during the initial 8 wk. and was essentially constant thereafter. The average daily increases in body weights for the aureomycin-fed (AA) and for the control (CC) groups were 2.0 and 1.7 lb., respectively. It is apparent, however, that the growth-stimulatory effect is dependent upon the continuous presence of the antibiotic in the diet, since after the aureomycin was omitted the accelerated rate of weight gains of calves in group AC ceased. This is in agreement with observations of Berg *et al.* (3) with chicks.

Introduction of aureomycin into the diet of calves not previously fed the antibiotic (group CA) resulted in an increase in feed (hay) consumption and a concomitant acceleration in rate of weight gain, suggesting that the latter response is dependent at least in part upon the former. This postulate is supported further by the reduction in feed intake and in rate of growth of calves in group AC following removal of aureomycin from the diet. The manner in which the antibiotic exerts its influence upon appetite, however, remains obscure. If the mode of action is "selective" bacteriostasis, it is possible that under certain conditions a more favorable bacterial flora in the gastrointestinal tract with subsequent improvement in health and vigor and an enhancement of appetite might result. Inasmuch as calves in group CA reacted favorably to aureomycin supplementation, it appears that a functional rumen at the time of introduction of the antibiotic into the diet of the dairy calf is not antagonistic to enhanced appetite and growth. The reason for the appetite stimulation resulting from aureomycin supplementation to dairy calves as contrasted to the appetite suppression observed in steers (2) and in lambs (4, 5, 6), however, is not clear.

The comparative changes in body measurements of calves in the various dietary groups were strikingly similar to the changes observed in body weights. The among-group differences were smallest for height at withers, the measurement which would be expected to estimate most accurately the changes in skeletal development. Moreover, the differences among groups were only slightly greater for chest depth and were largest for circumference of chest and circumference of barrel. Thus, although the greater weight gains of calves fed aureomycin may be due in part to more rapid skeletal development, improved condition (fleshing) of the animals also apparently is a contributing factor. Furthermore, since increases in feed consumption and in barrel circumference were greater among aureomycin-fed calves, differences in gastrointestinal volume may have been involved.

The supplemented calves received an average of approximately 7 mg. of aureomycin hydrochloride per pound of feed consumed, a level which apparently was adequate for marked growth stimulation. Whether a lower level would have been equally as effective cannot be ascertained on the basis of the present study. It is possible also that a higher antibiotic intake might have enhanced the responses. This seems unlikely, however, since four calves, not included in the data presented, that were fed 240 mg. aureomycin hydrochloride daily exhibited weight and body measurement changes similar to those of the supplemented calves (groups AA and CA).

Although the present experiment and previous studies demonstrate the growth-stimulating effect of aureomycin on dairy calves, many questions remain unanswered, including the possible effects of antibiotic feeding on the ultimate conformation and on the reproductive efficiency of the animals and on the effectiveness of future use (dietary and therapeutic) of antibiotics. Moreover, the comparative effects of various antibiotics need to be determined. Therefore, investigations designed to clarify some of these relationships have been initiated and will be reported later.

SUMMARY

Thirty-two dairy calves were divided at 116 days of age into four comparable groups. Calves in groups AA and AC previously had received crystalline aureomycin hydrochloride in the milk, whereas calves in groups CA and CC had been fed no antibiotic. During the subsequent 12-wk. period calves in groups AA and CA received aureomycin (Aurofac A in the concentrate mixture) and those in groups AC and CC were fed none.

During the 12-wk. experimental period, gains in body weight and increases in circumference of chest and of barrel were significantly greater ($P = 0.01$) for calves fed aureomycin (groups AA and CA) than for calves receiving no antibiotic (groups AC and CC). Differences in height at withers and in depth of chest were less.

Although previous treatment (groups AA and AC vs. CA and CC) had less effect than experimental treatment on changes in weight and in the various body measurements, the mean values in each instance were greater for calves not previously fed aureomycin.

Mean weights at the termination of the experiment were greatest for calves in group AA, followed in decreasing order by those in groups CA, AC and CC. With the exception of barrel circumference, the group mean values for the various body measurements ranked similarly. Hay and water consumptions were greater for calves fed aureomycin.

ACKNOWLEDGMENT

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REFERENCES

(1) Bartley, E. E., Fountaine, F. C., and Atkeson, F. W. The Effect of an APF Concentrate Containing Aureomycin on the Growth and Well-being of Young Dairy Calves. J. Animal Sci., 9: 646–647. 1950.

(2) Bell, M. C., Whitehair, C. K., and Gallup, W. D. The Effect of Aureomycin on Digestion in Steers. Proc. Soc. Exptl. Biol. Med., 76: 284–286. 1951.

(3) Berg, L. R., Bearse, G. E., McGinnis, J., and Miller, V. L. The Effect of Removing Supplemental Aureomycin from the Ration on the Subsequent Growth of Chicks. Arch. Biochem., 29: 404–407. 1950.

(4) Colby, R. W., Rau, F. A., and Couch, R. J. Effect of Feeding an "Animal Protein Factor" Concentrate to Young Lambs. Am. J. Physiol., 163: 418–421. 1950.

(5) Colby, R. W., Rau, F. A., and Dunn, R. C. Effect of Feeding Aureomycin to Fattening Lambs. Proc. Soc. Exptl. Biol. Med., 75: 234–236. 1950.

(6) Colby, R. W., Rau, F. A., and Miller, J. C. The Effect of Various Antibiotics on Fattening Lambs. J. Animal Sci., 9: 652. 1950.

(7) Haq, M. O., Rusoff, L. L., and Gelpi, A. J., Jr. Antibiotic Feed and Vitamin B_{12} Supplements for Lactating Dairy Cows. Science, 115: 215–216. 1952.

(8) Jacobson, N. L., Kaffenzakis, J. G., and Murley, W. R. The Response of "Ruminating" Dairy Calves to Aureomycin Feeding. J. Animal Sci., 10: 1050–1051. 1951.

(9) Loosli, J. K., and Wallace, H. D. Influence of APF and Aureomycin on the Growth of Dairy Calves. Proc. Soc. Exptl. Biol. Med., 75: 531–533. 1950.

(10) Murley, W. R., Jacobson, N. L., and Allen, R. S. The Effect of Aureomycin Supplementation on Growth and Feed Utilization of Young Dairy Calves. J. Dairy Sci., 35: 846–856. 1952.

(11) Rusoff, L. L. Antibiotic Feed Supplement (Aureomycin) for Dairy Calves. J. Dairy Sci., 34: 652–655. 1951.

(12) Rusoff, L. L., Davis, A. V., and Alford, J. A. Growth-promoting Effect of Aureomycin on Young Calves Weaned from Milk at an Early Age. J. Nutrition, 45: 289–300. 1951.

(13) Snedecor, G. W. *Statistical Methods.* 4th ed. The Iowa State College Press, Ames. 1950.

(14) Voelker, H. H., and Cason, J. L. Antibiotics Studies with Dairy Calves. J. Animal Sci., 10: 1065. 1951.

THE PRESERVING ACTION OF SUBTILIN AND MILD HEAT IN NORMAL AND CONCENTRATED MILK[1]

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The use of antibiotics in the preservation of food first was studied by Curran and Evans (8, 9), who reported that neither penicillin nor streptomycin was a reliable preserving agent for milk, although penicillin at extremely low levels was shown to be sporostatic and sporocidal against a wide range of organisms; the greater efficiency of these antibiotics when used in conjunction with mild heat was noted. Beginning late in 1949, a series of preliminary reports (2, 3, 4, 5) based upon the work of Anderson and Michener of the Western Regional Research Laboratory, revealed that subtilin when used in low concentration (10 to 20 ppm.) and combined with mild heating exerted a remarkable preserving effect in a variety of canned foods, including milk. Data (2) seemed to indicate that the spores of the most significant spoilage species, including *Clostridium botulinum*, were killed or effectively inhibited by the subtilin-heat treatment. These reports aroused wide interest in the press and canning industry, since it was recognized that such a procedure, if proved to be effective, safe and practicable, would make possible the preservation of low-acid canned foods without pressure heating.

The potential usefulness of the subtilin-heat process in the preservation of fluid and concentrated milk led us to investigate the effectiveness of the method. The results are reported in this paper.

MATERIALS AND METHODS

Spores of the aerobic species were produced on large bottle slopes of nutrient agar; after suitable incubation the crop was harvested and washed in the usual way. Clumps were broken up by shaking with small glass beads, after which the dispersed cells were stored in sterile distilled water at 6° C. until used. Anaerobic spores were produced in liver broth and used directly after concentration without washing. The milk, obtained from the Bureau of Dairy Industry experimental farm at Beltsville, Md., was fresh and of good quality. The tubed samples were heated in a thermostatically controlled glycerine bath.

EXPERIMENTS AND RESULTS

In the first experiment, raw whole milk was treated and stored in small lacquered cans of 150-ml. capacity. The technique was as follows: 46 to 49 ml. of chilled milk were measured into a series of sterile cans; to each was added, first, the inoculum, then the subtilin solution required to give the desired final concen-

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[1] This study was carried out in cooperation with the Bureau of Agricultural and Industrial Chemistry.

tration; 50 ml. more of milk then were added and the cans sealed, shaken vigorously for several minutes and placed in a pilot sterilizer which rotated the samples slowly in water heated by steam. The cans remained in the sterilizer for 10 to 13 min. after their internal temperature reached 100° C.; they then were plunged into ice water, agitated briskly and placed in storage at 30° C. (exceptions noted). The results (table 1) show that several cultures at 20 ppm. and two at 40 ppm. subtilin induced spoilage in the milk over a 5-mo. storage period. The inoculum in some samples was greater than would be normally encountered; in others, it was not greatly in excess of levels which occasionally are attained in commercial practice.

The influence of size of inoculum upon the preserving action was next investigated. In this experiment, 10-ml. quantities of milk were dispensed into 125-ml. Erlenmeyer flasks and sterilized by autoclaving; after chilling to 0 to 6° C., the

TABLE 1

Influence of subtilin and heat[a] upon the preservation of canned whole milk artificially inoculated with spores of miscellaneous aerobic and anaerobic species

Organism	Viable spores/ml. in milk before heating	Subtilin (ppm.) in milk before heating			
		0	10	20	40
B. subtilis (9499)	36,000,000	+[b]	+4	+5	+16
B. subtilis (6)	100,000,000	+4	+6	+	−
B. subtilis (LB)	1,180,000	+	+	+	
B. aterrimus (230)	650,000	+	−[c]	−	
B. brevis (Temple)	85,000	+	−	−	
B. megatherium	120,000	+	+	−	
B. metiens	2,000	+	−	−	
B. stearothermophilus (26)[d]	218,000	+	−	−	
Cl. thermosacchrolyticum (TA-3814)[d]	216,000	+	−	−	
P. A. 3679	60,000	+4	+9	+20	+30

[a] 100° C.—10 min.
[b] + = spoilage and day in storage it occurred.
[c] − = no change.
[d] Storage temp., 45° C.; all others 30° C. Storage period, 5 mo.

milk was seeded with the spores of *Bacillus subtilis* and mixed by gentle rotation of the flasks; subtilin then was added, sufficient to give a final concentration of 20 ppm. and the flasks again rotated to insure uniform distribution. The samples were quickly transferred to sterile tubes and heated at 100° C. for 10 min., cooled and stored at 30° C. The results are shown in table 2. All samples containing 28,000 or more spores per milliliter ultimately spoiled, although in all except those with the highest inoculum, a delay in the development of spoilage over the control is evident. Samples containing 2,800 or less spores per milliliter remained unchanged for a period of 3 mo. and heating in the absence of subtilin protected the sample containing an average of 0.28 spores per milliliter (2 to 3 spores per sample).

The greatest hazard to health in low-acid canned foods is presented by *Cl. botulinum*, which, if not completely destroyed by the processing treatment, may elaborate a deadly toxin in the subsequent storage of the product. To test the

activity of subtilin against this organism, raw whole milk was inoculated with viable spores, treated with subtilin and stored in small cans (150-ml. capacity). The technic was similar to that described in experiment 1. Table 3 shows the results obtained at different levels of subtilin and spore concentration. It is clear

TABLE 2

The preservation of milk by subtilin (20 ppm.) and heat (100° C.—10 min.) as influenced by concentration of spores[a]

Viable spores/ml. in milk before heating	After storage[b] for 3 mo.		
	Spores only	Spores and heat	Spores, heat and subtilin
28,000,000	+4[c]	+4	+ 5
2,800,000	+4	+4	+ 9
280,000	+4	+4	+15
28,000	+4	+4	+15
2,800	+4	+4	–
280	+4	+4	–
28	+4	+9	–
2.8	+4	+4	–
0.28	+4	–[d]	–

[a] *B. subtilis* (9499)
[b] Storage temp., 30° C.
[c] + = spoilage and day in storage it occurred.
[d] – = no change.

that this, the most significant of all spoilage species, is not effectively controlled by subtilin, even in high concentration. One hundred ppm. of the antibiotic, in combination with mild heating, were insufficient to prevent spoilage when the spore inoculation was relatively high. With much fewer spores the samples did not spoil with 40 ppm. of subtilin; however, in all the unspoiled samples which

TABLE 3

The influence of subtilin and heat[a] *upon the preservation*[b] *of canned whole milk artificially inoculated with spores of Clostridium botulinum (62A)*

Viable *Cl. botulinum* spores/ml. in milk before heating	Subtilin (ppm.) in milk before heating			
	0	40	80	100
1,120,000	+[c]	+	+	+
120,000	+	+	+	
12,000	+	–[d]	–	
1,200	+	–	–	–
120	+	–	–	–
0	+	–	–	–

[a] 100° C.—10 min.
[b] Storage period, 5 mo. at 30° C.
[c] + = typical *Cl. botulinum* spoilage, including coagulation and peptonization.
[d] – = no change.

initially received 1,000 or more spores per milliliter, viable spores could be demonstrated after 5 or 6 mo. of storage; thus, the low-spore samples, which showed no change within 5 mo. are potentially subject to spoilage and toxin formation at a later period. The samples receiving minimal inoculation and 100 ppm. of subtilin and heat were practically all spoiled after 15-mo. storage.

In the last experiment, the preserving action of subtilin and mild heat was observed in uninoculated whole and evaporated milk. The milk was treated and stored in small cans according to the method previously described. The evaporated milk contained 26 per cent solids and was homogenized at 2,500 lb. per in.^{2} prior to canning and treatment. The aerobic plate count of the unconcentrated milk before treatment, after heating at 85° C. for 15 min., varied from 1 to 410 per milliliter (spores); similarly, in the evaporated milk, the count ranged from 260 to 2,400 per milliliter (spores).

Table 4 shows some results obtained with subtilin-processed uninoculated

TABLE 4

Influence of subtilin and heat upon the preservation of canned uninoculated normal and evaporated milks[a]

Subtilin	Heat treatment	Whole milk			Evaporated milk		
		No. of expts.	After storage for 5–8 mo.		No. of expts.	After storage for 6 mo.	
			good	bad		good	bad
(*ppm.*)			(%)	(%)		(%)	(%)
0	100° C.—10 min.	14	0	100	6	0	100
10	"	"	86	14	.....	.....	.....
20	"	"	88	12	6	9	91
30	"	"	92	8	.....	.....	.....
40	"	"	91	9	6	18	82
50	"	"	100	0	.....	.....	.....
60	"	.....	.....	.....	6	37	63
100	"	.....	.....	.....	6	75	25
0	100 ° C.—20 min.	2	50	50	2	0	100
10	"	2	100	0	.....	.....	.....
20	"	.....	.....	.....	2	10	90
30	"	2	100	0	.....	.....	.....
50	"	2	100	0	.....	.....	.....
60	"	.....	.....	.....	2	42	58

[a] Milk stored in sealed cans (100 ml./can); incubation temp., 30° C.

whole and evaporated milk. Most of the whole milk samples remained bacteriologically unchanged for 5 to 8 mo.; these samples contained very few heat-resistant spores, usually less than 30 per milliliter. When some of the whole milk was divided and a portion processed after passing over a cooler and through the homogenizer, most of the samples spoiled, owing to the resulting small build-up in the resistant spore population of the samples. Some tests were carried out below 100° C. down to that of pasteurization; most of the latter underwent spoilage of mold origin or non-bacterial enzyme digestion. Subtilin processing was singularly ineffective in preserving uninoculated evaporated milk even with high concentrations of the antibiotic. This is due, in part, to the increased spore content of the milk derived from the plant equipment (cooler, vacuum pan, homogenizer, etc.) and, in part, to the effect of increased milk solids *per se*, some constituents of which apparently have a neutralizing effect upon the subtilin.

DISCUSSION

The foregoing observations show that subtilin-heat treatment, as described by Anderson and Michener, is not an effective method for preserving normally produced whole or concentrated milk. Results reveal that the process does not control significant spoilage-inducing species at high subtilin levels, and the treatment fails to control the growth of the widely disseminated *Cl. botulinum*, the most dangerous of all food poisoning organisms. For these reasons, subtilin processing cannot be safely used for the preservation of milk for human consumption. Fluid whole milk, if carefully protected from significant spore contamination, usually can be preserved without bacteriological change for long periods by the subtilin-heat process. Within this limitation, it provides a useful method for the study of physico-chemical changes incident to long storage, the advantage of the method deriving from the fact that the treatment produces little apparent alteration in the properties of the milk.

Bacterial species differ in their susceptibility to the combined action of subtilin and heat and in accord with usual experience the effectiveness of a given treatment is correlated with the spore load. For this reason relatively heavy spore inocula must be employed in the evaluation of sterilizing processes, since it is not only the normal but the unusual or extreme conditions of general practice which must be insured against.

The initial subtilin-heat treatment greatly reduces the number of spores that can be demonstrated by the usual methods of subcultivation and their number in the absence of germination decreases with prolonged storage; the fact that many spores survive late in storage, yet within this period fail to germinate, suggests that subtilin retains some of its sporostatic activity for a long time.

Before this study was completed additional reports appeared in the literature dealing with the use of subtilin in food materials other than milk, Cameron (6), Olcott (10), Adams *et al.* (1), Williams and Campbell (11) and Cameron (7); these including Olcott's report on further observations of Anderson and Michener are in essential agreement with those reported in this paper.

SUMMARY

Normal and concentrated milks, with and without artificially added resistant bacterial spores, were processed with subtilin and heat at 100° C. for 10 min. and observed for spoilage over a period of 5 to 8 mo. Spoilage occurred consistently in milk inoculated with commercially important organisms, including *Cl. botulinum*, at relatively high subtilin levels. The preserving efficiency of the subtilin-heat treatment was correlated with the spore load and the degree of concentration (per cent of milk solids). It is concluded that the subtilin-heat process is not a safe or effective method for preserving normally produced whole or concentrated milk.

REFERENCES

(1) ADAMS, A. T., AYERS, J. C., AND TISCHER, R. G. The Effect of Subtilin and Heat in Preventing Spoilage of Comminuted Beef. Food Technol., 5: 82. 1951.

(2) ANDERSON, A. A., AND MICHENER, H. D. Effect of Subtilin on Bacterial Spores. Bact. Proc., 28–29. 1950.

(3) ANDERSON, A. A., AND MICHENER, H. D. Preservation of Foods with Antibiotics. I. The Complementary Action of Subtilin and Mild Heat. Food Technol., 4: 188–189. 1950.

(4) ANONYMOUS. U. S. Dept. Agr. Press Release. Antibiotics Tested for Preserving Food. (USDA 2765-49), Dec. 29, 1949.

(5) ANONYMOUS. U. S. Dept. Agr. Press Release. Research shows Antibiotic Kills Food Organism. (USDA 1216-50), May 17, 1950.

(6) CAMERON, E. J. Association Issues Press Release on Subtilin. Nat. Canners Assoc. Information Letter no. 1305, 287. 1950.

(7) CAMERON, E. J. Use of Antibiotics in Preserved Foods. Proc. Conf. on Research, American Meat Inst., 3: 32–38. 1951.

(8) CURRAN, H. R., AND EVANS, F. R. The Activity of Penicillin in Relation to Bacterial Spores and the Preservation of Milk. J. Bact., 52: 89–98. 1946.

(9) CURRAN, H. R., AND EVANS, F. R. The Activity of Streptomycin in Relation to Bacterial Spores and the Preservation of Milk. J. Bact., 52: 142. 1946.

(10) OLCOTT, H. S. Problems in the Application of Antibiotics to Food Processing. Nat. Canners Assoc. Information Letter no. 1325, 102. Feb. 28, 1951.

(11) WILLIAMS, O. B., AND CAMPBELL, L. L., JR. The Effect of Subtilin on Thermophilic Flat Sour Bacteria. Food Research, 16: 347–352. 1951.

ANTIRACHITIC ACTIVITY OF SOME DEHYDRATED ALFALFA HAYS AND THE EFFECT OF ADDING LIMESTONE TO THE GRAIN RATION ON THE DEVELOPMENT OF RICKETS IN CALVES

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There has been a large increase in the production and feeding of alfalfa and grasses cured with little or no exposure to the vitamin D-activating rays of the sun, and various commercially dehydrated alfalfa products also are now being sold widely for animal feeds. Some of these crops, especially the dehydrated alfalfa products, are cut at a very immature stage.

It seems desirable, therefore, to know the antirachitic properties of such feeds when they are used as the only source of vitamin D for young calves.

Studies already have been reported by the Bureau of Dairy Industry which showed that alfalfa, which was barn-dried immediately after cutting, and alfalfa silage protected calves against the development of rickets (16) and that barn-dried alfalfa hay and wilted alfalfa silage cured rickets in calves (12). Other investigators have shown that corn silage cured rickets in calves (5). The vitamin D activity, or antirachitic activity, of an alfalfa crop as it stands in the field was shown to vary with the stage of maturity (16).

In these previous studies (12, 16) the grain mixture contained 2 per cent ground limestone. Some investigators have reported that additions of minerals or calcium aid in the cure or prevention of rachitic symptoms (2, 6, 9, 10, 13), while other investigators have observed little or no beneficial effect (3, 8, 18). Therefore, it seemed desirable to determine whether additions of limestone to the grain ration would modify the antirachitic effect of the dehydrated forages used in the investigation reported here.

EXPERIMENTAL

Three different dehydrated hays were used. They were: (a) a third cutting of alfalfa grown at Beltsville, Md., in 1948, and dehydrated in a portable, pneumatic, double-pass dehydrator; (b) a commercially dried and pelleted alfalfa produced in 1948; and (c) a similar product produced in 1949. Samples of each were composited as fed for determination of its vitamin D content by the rat curative line test procedure (17) and for calcium and phosphorus analysis. The calcium content was 1.192, 1.464 and 1.294 per cent and the phosphorus content was 0.334, 0.29 and 0.284 per cent, respectively, for the three hays on the dry matter basis.

The grain mixture used has been given previously (16) and in this experiment it contained 0, 2 or 4 per cent of ground limestone. The average values for the three grain mixtures were 0.236, 0.901 and 1.566 per cent calcium and 0.822, 0.793 and 0.775 per cent phosphorus of the dry matter, respectively. These values

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and average published values for dried beet pulp and milk were used in calculating the calcium and phosphorus intakes of the calves as given in table 3.

Six groups of calves were used in this work. The calves in groups 1 to 4, inclusive, received dried beet pulp as their only roughage. Those in groups 1 and 2 were negative controls with 0 and 4 per cent limestone in their grain mixture, respectively, receiving no vitamin D. Those in groups 3 and 4, positive controls, received vitamin D as viosterol at 3,000 I.U. per hundredweight or ten times the minimum requirement (3) and no limestone in their grain mixture. For comparative purposes, group 3 was slaughtered at the same age as the negative control calves, and group 4 was slaughtered at the same age (240 days) as those in the alfalfa groups. Calves in group 5 received the hay grown and dehydrated at Beltsville, and calves in group 6 received the commercially dehydrated and pelleted alfalfa. Since the results on the calves fed the two pelleted commercial products generally were indistinguishable, and because of the small numbers, the calves fed these two products have been considered as one group. Some calves in each of the two hay groups received 0, 2 and 4 per cent limestone in their grain mixture. These were designated as sub-groups A, B and C, respectively, in groups 5 and 6.

Calves were fed the hays beginning at 10 days of age. Roughage intake, hay or dried beet pulp, was limited to 1.5 lb. per hundredweight when this rate of intake was reached. Very young calves did not eat the pellets satisfactorily unless they were partially broken up by putting them through a hammer mill with the screens removed. The calves were Jersey and Holstein males and always were kept in a darkened barn. Further details of care, procedure and methods used have been mentioned previously (16).

Periodic X-ray negatives of the radius and ulna, periodic calcium, inorganic phosphorus and phosphatase values of the blood, terminal bone ash content and the appearance of visible and clinical rachitic symptoms by procedures previously described (12, 15, 16) were used to estimate the antirachitic effects of the rations used in this experiment.

RESULTS AND DISCUSSION

Antirachitic value of dehydrated hays for calves. Growth rates of all calves were comparable until they were about 60 days of age (table 1). After this time, the rate of gain of the negative control calves (groups 1 and 2) decreased. Visible signs of rickets began to appear in these calves between 70 and 90 days of age, which is similar to previous observations (16). These animals were slaughtered when movement became limited and before serious anorexia developed. Average daily gain, during the experiment, of the negative control calves was significantly below ($P < 0.01$) that of any other group. The body weight gains of calves fed alfalfa grown and dehydrated at Beltsville (group 5-A, B and C), the commercially dehydrated and pelleted alfalfa (group 6-A, B and C), and those fed viosterol in group 4 were practically identical (table 1).

The average calcium, phosphorus and phosphatase values found in the blood of calves on various dehydrated hays compared to positive and negative control

calves are shown by monthly intervals in figure 1. This figure also includes data from a past experiment which is described later. Ten calves receiving the commercially dehydrated and pelleted alfalfa (group 6) had lower plasma inorganic phosphorus and calcium levels and higher phosphatase values than calves in the other hay groups after the first 3 mo. on the experimental diets. Eight calves fed the 1948 Beltsville dehydrated alfalfa (group 5) and 11 calves fed field-cured and barn-dried hays and silage that protected them from all rachitic symptoms in a previous experiment (16), were very near the same in the three blood constituents, as shown in figure 1. They both compare favorably with the positive control calves fed beet pulp and viosterol.

TABLE 1

Data on the feeds fed to the 6 groups of calves and average values (with their standard deviations) for the criteria used to evaluate the extent of rickets in the calves

Group no.	1	2	3	4	5 A B & C	6 A B & C
No. of calves	7	4	3	3	8	10
Roughage	Dried beet pulp	Dried beet pulp	Dried beet pulp	Dried beet pulp	Dehyd. alfalfa	Dehyd. pellets
Source of vitamin D	none	none	viosterol	viosterol	"	"
Limestone in grain (%)	0	4	0	0	0–2 & 4	0–2 & 4
Age at slaughter (*d.*)	140	148	142	230	235	240
	±11	±20	±13	±24	±7	±5
Av. daily gain (*lb./day*)						
0–60 d.	0.78	0.67	0.78	0.81	0.83	0.81
	±0.24	±0.02	±0.20	±0.23	±0.23	±0.20
Birth to slaughter	0.79	0.74	1.18	1.30	1.34	1.32
	±0.23	±0.16	±0.41	±0.16	±0.24	±0.19
Birth weight (*lb.*)	70	62	62	68	71	66
Grain consumed (*lb.*)	171	165	214	547	646	610
Roughage consumed (*lb.*)	137	148	159	497	603	648
Skimmilk consumed (*lb.*)	1004	1032	933	1091	1127	967
Kidney size						
(*% of body weight*)	0.507	0.575	0.382	0.333	0.342	0.386
	±.08	±.08	±.06	±.08	±.03	±.04
Bone ash (%)	32.66	34.73	50.50	51.63	51.63	46.10
	±4.1	±2.2	±0.7	±1.8	±3.2	±2.2
Terminal ulnar epiphyseal						
cartilage width (*mm.*)	7.8	5.8	2.2	0.5	0.7	1.3
	±2.8	±1.9	±0.6	±0.7	±0.4	±0.7
Rate of closure of cartilage						
(*mm./d.*)	−0.014	0.0	+0.043	+0.025	+0.030	+0.024
	±.02	±.04	±.007	±.004	±.004	±.003

The values shown in figure 1 are for a total of ten positive control calves from group 3 and group 4 of this experiment combined with the positive control group from the previous experiment (16). The values for the positive control calves (fig. 1) thus are a composite of two experiments carried out under identical conditions and should prove useful as a comparison or guide for values found under other conditions. Similarly, the values shown in figure 1 for 14 negative control calves are an average of groups 1 and 2 of this experiment and the negative controls of the former experiment (16).

Average plasma calcium values from the 180th to the 240th day of age for the calves receiving the dehydrated-pelleted alfalfa were lower than for any of the other normal groups shown. The differences were highly significant ($P > 0.01$).

Average plasma inorganic phosphorus values for this period were highest for those 11 calves fed the various hays in the previous experiment. They were significantly higher ($P = 0.05$) than for the calves in either of the hay groups (5 or 6) of this experiment, but none of the hay groups was significantly different from the values for the positive control calves. For the same period, average serum

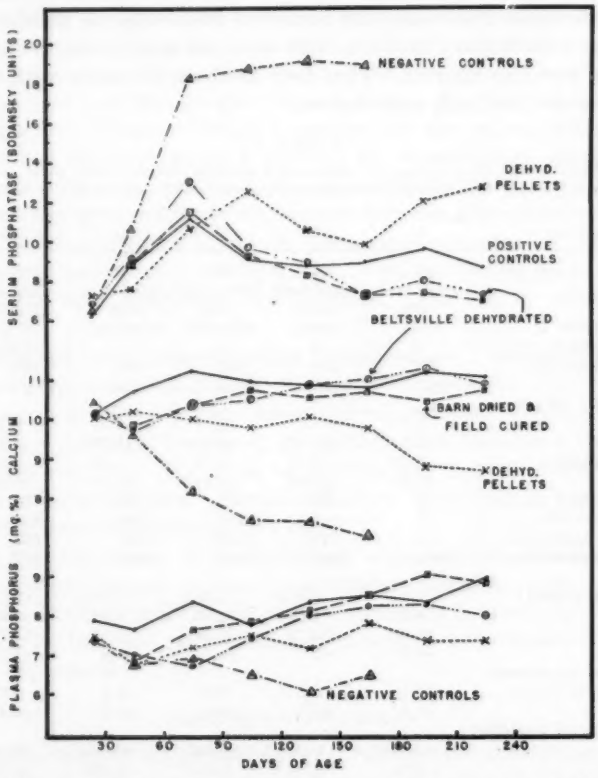

FIG. 1. Average plasma inorganic P, Ca and serum phosphatase concentrations at monthly intervals of calves receiving various alfalfa hays as their only source of vitamin D compared to positive and negative control calves. See text for further details.

phosphatase values of calves receiving the dehydrated-pelleted alfalfa were increased above the value for the calves fed either of the Beltsville hays (figure 1). The differences were highly significant ($P < 0.01$). They were significantly higher ($P = 0.05$) than the values for the positive control group.

The average values at 60 to 120 days of age for the negative control calves were significantly different ($P < 0.01$) from the values for any of the other four groups shown in figure 1. At this time the calves fed pellets also were lower in plasma calcium concentration than either of the other hay groups or the positive

control group ($P < 0.01$ or < 0.05). The positive control group was higher in plasma calcium and phosphorus at this age interval than those in the two groups fed alfalfa grown and processed at Beltsville, and in some comparisons this difference was significant, although the importance of it is not evident.

The data on these blood constituents indicated that the calves receiving the commercially dehydrated-pelleted alfalfa as their only source of vitamin D had some degree of clinical rickets during the last half of this experiment.

The average rib-end ash content given in table 1 for the calves receiving the dehydrated-pelleted alfalfa was significantly lower ($P < 0.01$) when the ash content found for calves in any of groups 3, 4 and 5 of this experiment and those considered normal in a previous experiment (16). The bone ash of rachitic calves (group 1 plus group 2) was significantly below ($P < 0.01$) that of any of the other groups.

The average ulnar epiphyseal cartilage width (15) at slaughter time of the calves fed the dehydrated-pelleted alfalfa (group 6) was wider than that of groups 4 or 5 (table 1). Individual variation was large and the differences were not statistically significant. The width of the ulnar epiphyseal cartilage of negative control calves at slaughter was significantly wider ($P < 0.01$) than that of calves in any other group at about 140 days of age.

The rate of epiphyseal cartilage closure to slaughter time (15) of calves receiving dehydrated-pelleted alfalfa was significantly less ($P < 0.02$) than that of normal calves. The negative control calves on an average had a negative rate of closure, and this was significantly different ($P < 0.01$) from any of the other groups at the same age (table 1).

The size of the kidney expressed as a percentage of the body was larger for the calves receiving the dehydrated-pelleted alfalfa than for the calves in groups 3, 4 or 5 (table 1). This difference was statistically significant ($P = 0.05$). The negative control calves in this experiment (groups 1 and 2) and rachitic calves in the previous experiment (16) had enlarged kidneys. This difference in kidney size between negative controls and any other group was highly significant ($P < 0.01$).

At slaughter time most of the calves receiving dehydrated-pelleted alfalfa had bones that were easier to cut with a knife and to saw through than were the bones of the calves in groups 3, 4 or 5. Some of these calves in group 6 also had some degree of enlargement of the rib-ends. Visible symptoms of rickets were noticed in some calves receiving the commercially dehydrated-pelleted product with no limestone in their grain ration, while calves receiving the same hays but with limestone added to their grain ration showed no visible symptoms of rickets.

Calves in groups 1 and 2 had soft bones at slaughter time and showed all the visible symptoms of rickets and the post-mortem findings indicative of rickets that have been mentioned previously (3, 4, 5, 10, 16).

These data indicate that visible signs of rickets, such as stiffness, bowing of front legs, rib-end enlargement and straightness of the pasterns may appear in calves fed commercially dehydrated-pelleted alfalfa as their only source of vitamin D. Clinical symptoms such as increased serum phosphatase, decreased

serum calcium, delayed epiphyseal closure and reduced bone ash values may occur more frequently. These observations suggest that under these conditions early cut-dehydrated alfalfa contains insufficient vitamin D to meet the needs of growing dairy calves. On the other hand and in confirmation of previous findings (16), dehydrated hay cut at the hay stage and grown at Beltsville may prevent both visible and clinical rachitic symptoms from developing when fed to calves at 1.5 lb. per hundredweight.

Effect of varying the calcium content of the grain ration. In order to see if the amount of calcium in the ration affected the response of the calves under the conditions of this experiment, those calves receiving no limestone, groups 5-A

TABLE 2

Effect of adding limestone to the grain mixture on the various criteria used to evaluate rickets in rachitic and non-rachitic calves and those receiving dehydrated alfalfa

	Calves fed dehydrated alfalfa with		Negative control calves		Positive control calves	
	No limestone in grain	2 or 4% limestone in grain	No limestone in grain	4% limestone in grain	No limestone in grain	2% limestone in grain plus minerals
Group no.	5-A, 6-A	5-B, 5-C 6-B, 6-C	1	2	4	(16)[b]
Av. bone ash (%)	46.0	50.3	32.7	34.7	51.6	53.2
Ulnar epiphyseal cartilage width (*mm.*)	1.46	0.84	7.8	5.8	0.50	0.64
Av. plasma Ca (*mg. %*)[a]	9.21	10.33	7.02	8.66	11.17	11.02
Av. serum phosphatase (*Bodansky units/100 ml.*)[a]	11.5	9.2	18.0	17.4	8.8	9.4
Kidney weight (*% of body weight*)	0.375	0.363	0.507	0.575	0.333	0.312
Visible evidence of rickets	only in group 6-A	none	yes	yes	none	none
Post-mortem evidence of rickets	only in group 6-A	some in group 6	yes	yes	none	none

[a] Av. during 2-mo. period preceding slaughter.

[b] Positive control group no. 5 from previous experiment. (See J. Dairy Sci., 34: 920. 1951.)

and 6-A, were compared to calves receiving limestone in the grain, groups 5-B, 5-C, 6-B and 6-C. Average data on this comparison and for negative and positive control calves not receiving and receiving added limestone are shown in table 2.

The addition of limestone to the ration of positive and negative control calves produced small but statistically insignificant changes with the criteria used to evaluate rickets in calves. In the negative control calves, the average values for bone ash, plasma calcium concentration and ulnar epiphyseal cartilage width and its rate of closure showed a slight alleviation of the rachitic condition in the calves receiving the limestone. This slight effect was caused by a two-fold difference in calcium intake and Ca : P ratio (table 3).

The rapid development of severe rickets in negative control calves at about 4 mo. of age may have been due partly to the removal of milk from the diet at this age. This caused a reduction in calcium and phosphorus intakes in both positive and negative control calves. The intakes by monthly ages are given in table 3.

The positive control calves receiving the extra limestone and bone meal (16) had a slightly higher bone ash content than those without added minerals (table 2), but the difference was not statistically significant. Other criteria used to evaluate rickets showed no differences between those two positive control groups, yet they had about a two-fold difference in calcium intakes and Ca : P ratio.

TABLE 3

Average calcium and phosphorus intakes by monthly ages (mg./lb. body weight/day)

Group	Age (*d.*)								Limestone in grain
	11–30	31–60	61–90	91–120	121–150	151–180	181–210	211–240	
Calcium									(%)
1	66	89	88	64	47	.....	.....	.....	0
2	99	156	163	145	133	.....	.....	.....	4
3 & 4	70	87	94	68	55	54	52	48	0
5-A & 6-A	76	122	135	116	109	112	108	108	0
5-B & 6-B	88	139	160	147	157	154	145	146	2
5-C & 6-C	109	193	212	214	217	210	201	195	4
Phosphorus									
1	59	94	86	67	55	.....	.....	.....	0
2	64	88	88	61	55	.....	.....	.....	4
3 & 4	64	91	93	72	64	58	56	55	0
5-A & 6-A	67	104	111	95	88	85	80	75	0
5-B & 6-B	70	103	109	93	92	89	81	79	2
5-C & 6-C	66	100	104	91	84	79	75	73	4
Calcium : Phosphorus ratio									
1	1.1 : 1.0	1.0 : 1.0	1.0 : 1.0	1.0 : 1.0	0.9 : 1.0	.....	.....	.....	
2	1.5	1.8	1.8	2.1	2.4	.....	.....	.....	
3 & 4	1.1	1.0	1.0	1.0	0.9	0.9	0.9	0.9	
5-A & 6-A	1.1	1.2	1.2	1.2	1.2	1.3	1.4	1.4	
5-B & 6-B	1.3	1.4	1.5	1.6	1.7	1.7	1.8	1.9	
5-C & 6-C	1.7	1.9	2.0	2.3	2.6	2.7	2.7	2.7	

The calves that received the various dehydrated alfalfas with extra limestone in their grain showed, on an average, increased values for bone ash, plasma calcium and decreased values for serum phosphatase, ulnar epiphyseal cartilage width and kidney size compared to calves not receiving limestone. The difference in bone ash was significant ($P = 0.04$). Because of the variation caused by the dehydrated hays, the other average differences due to level of calcium intake were not statistically significant by the *t* test (14).

The addition of 2 and 4 per cent limestone to the grain fed caused about a 130 and 190 per cent increase, respectively, in both calcium intake and Ca : P ratio. Under certain conditions other investigators have found an increased

calcium intake to have some beneficial action on the alleviation of the rachitic condition (2, 6, 9, 10, 13).

It is evident that the effect produced by adding 4 per cent limestone to the grain mixture, thus doubling the calcium intake of the basal ration, was small compared to the effect of substituting for the dried beet pulp alfalfa that had been cured by any of the methods studied. When the vitamin D intake was inadequate, as in group 6 (or group 2), then the addition of limestone had only a small alleviating effect on the development of clinical rickets and bone ash values. When the vitamin D intake was minimal as in group 5, the addition of limestone to the grain mixture increased ($P = 0.03$) the ash content of the bone. However, when the vitamin D intake was adequate, as in the positive control groups, the added bone meal and limestone had little, if any, effect. Thus, the alleviating effect of added calcium intake on ash content of the bone was influenced by the vitamin D content of the diet being more pronounced when the vitamin D intake was near requirement. This effect was not statistically significant, although it was observed at both higher and lower vitamin D intake levels.

TABLE 4

Vitamin D content of hays used and the amount of vitamin D they supplied to calves when fed at the rate of 1.5 lb./ cwt.

Group no.	Source of hay	Vitamin D content	Vitamin D intake of calves	Presence of rickets
		(*I.U./lb.*)	(*I.U./cwt.*)	
5	Beltsville, dehydrated, 1948	101	150	None
6	Commercial, dehydrated-pelleted, 1948	56	84	Yes
6	Commercial, dehydrated-pelleted, 1949	< 40	60	Yes

The vitamin D intake and requirement of calves. The vitamin D content of the hays was determined by the rat-curative line test procedure (16, 17) and the results are presented in table 4. The Beltsville product contained about twice as much vitamin D as did the commercially dehydrated-pelleted products. However, this Beltsville product contained straw, weeds and grass as foreign material.

Samples of two other similar Beltsville products, but free of foreign material, contained 60 and 96 I.U. per pound. The amount of foreign material in the alfalfa probably may be another factor affecting the vitamin D potency of the hay crops in addition to the stage of maturity (12, 16).

The calves were fed the dehydrated alfalfa hays at the rate of 1.5 lb. per hundredweight, and this figure then was used in calculating their vitamin D intake, which is given in table 4. The calves on the lower intakes (group 6) showed signs of clinical rickets and some showed visible symptoms. Those receiving 150 I.U. per 100 lb. (group 5) were adequately protected from developing rickets, provided that the grain contained added limestone. Previously, we had observed that calves receiving 144 I.U. per 100 lb. showed slight clinical signs of rickets, while calves receiving 200 I.U. per 100 lb. were free from all rachitic symptoms (16). Apparently the absolute minimum vitamin D requirement for

young growing calves is about 150 I.U. per 100 lb. when supplied in the form of alfalfa hay. For comparison, the minimum vitamin D requirement for young calves was determined to be about 300 I.U. per 100 lb. when supplied as viosterol in addition to a basal ration (3), and 350 I.U. was adequate when the source was milk (11). The minimum vitamin D requirement of young sheep was found to be 180 to 200 I.U. per 100 lb. (1, 7). Our data with calves and that of Bechtel *et al.* (5) indicate that 150 to 200 I.U. per 100 lb. in the form of alfalfa or corn silage is adequate for young calves.

SUMMARY

The antirachitic potency of three dehydrated alfalfas has been studied with young dairy calves where the hay supplied the only source of the vitamin. The hay was fed to the calves at the rate of 1.5 lb. per hundredweight.

Data on blood levels of calcium, inorganic phosphorus and phosphatase, ulna epiphyseal cartilage width obtained by periodic x-ray examinations, rate of growth, terminal rib ash content and the appearance of visible and post mortem signs of rickets were used to evaluate the rachitic condition of all calves in the experiment.

One dehydrated hay supplying the calf with about 150 I.U. of vitamin D per 100 lb. of body weight was adequate when the grain ration contained added limestone, while one hay supplying 84 I.U. per 100 lb. body weight did not prevent the development of clinical rickets. The other hay provided less than 64 I.U. per 100 lb. and allowed visible and clinical rachitic symptoms to develop. Apparently, alfalfa cut at an early stage of maturity and then dehydrated probably will not itself supply a sufficient amount of vitamin D to prevent rickets in young calves.

By comparing 0 and 4 per cent limestone in the grain mixture, which about doubled the calcium intake, it was shown that added calcium had a definite but limited alleviating action on the development of rachitic conditions in calves.

REFERENCES

(1) Andrews, E. D., and Cunningham, I. J. The Vitamin D Requirement of the Sheep. New Zealand J. Sci. Technol., 27: 223–230. 1945.

(2) Auchinachie, D. W., and Fraser, A. H. H. The Effect of Lime and Cod Liver Oil on Sheep Fed a Calcium-deficient Ration. J. Agr. Sci., 22: 560–575. 1932.

(3) Bechdel, S. I., Hilston, N. W., Guerrant, N. B., and Dutcher, R. A. The Vitamin D Requirement of Dairy Calves. Pa. Agr. Expt. Sta. Bull. 364. 1938.

(4) Bechdel, S I., Landsburg, K. G., and Hill, O. J. Rickets in Calves. Pa. Agr. Expt. Sta. Bull. 291. 1933.

(5) Bechtel, H. E., Huffman, C. E., Duncan, C. W., and Huppert, C. A. Vitamin D Studies in Cattle. IV. Corn Silage as a Source of Vitamin D for Dairy Cattle. J. Dairy Sci., 19: 359–372. 1936.

(6) Carttar, M. S., McClean, F. C., and Urist, M. R. The Effect of the Calcium and Phosphorus Content of the Diet upon the Formation and Structure of Bone. Am. J. Path., 26: 307–331. 1950.

(7) Duckworth, J., Godden, W., and Thomson, W. The Relation between Rates of Growth and Rickets in Sheep on Diets Deficient in Vitamin D. J. Agr. Sci., 33: 190–196. 1943.

(8) FITCH, L. W. N., AND EWER, T. K. The Value of Vitamin D and Bone-flour in the Prevention of Rickets in Sheep in New Zealand. Australian Vet. J., **20**: 220–226. 1944.

(9) FRANKLIN, M. C., REID, R. L., AND JOHNSTONE, I. L. Studies on Dietary and Other Factors Affecting the Serum-calcium Levels of Sheep. Parts I to VI. Council for Scientific and Industrial Research Bull. 240. 1948.

(10) GULLICKSON, T. W., PALMER, L. S., AND BOYD, W. L. A Rickets-like Disease in Young Cattle. Minn. Agr. Expt. Sta. Tech. Bull. 105. 1935.

(11) HUFFMAN, C. F., AND DUNCAN, C. W. A Preliminary Study of the Vitamin D Requirement of Calves when Fed Natural Milks as the Source of this Vitamin. J. Dairy Sci., **18**: 432. 1935.

(12) MOORE, L. A., THOMAS, J. W., JACOBSON, W. C., MELIN, C. G., AND SHEPHERD, J. B. Comparative Antirachitic Value of Field-cured Hay, Barn-dried Hay, and Wilted Grass Silage for Growing Dairy Calves. J. Dairy Sci., **31**: 489–499. 1948.

(13) MIGOCOWSKY, B. B., AND EMSLIE, A. R. G. Interaction of Calcium, Phosphorus and Vitamin D. I. Influence of Dietary Calcium and Phosphorus on Body-weight and Bone Ash of Chicks. Arch. Biochem., **13**: 183. 1947.

(14) SNEDECOR, G. W. *Statistical Methods.* 4th ed. 1948. The Iowa State College Press, Ames.

(15) THOMAS, J. W. The Use of X-ray Photographs in the Detection of Rickets in Calves. BDIM-Inf-95. June 1950. J. Dairy Sci., **33**: 387. 1950, and unpublished data 1951–52.

(16) THOMAS, J. W., AND MOORE, L. A. Factors Affecting the Antirachitic Activity of Alfalfa and its Ability to Prevent Rickets in Young Calves. J. Dairy Sci., **34**: 916–928. 1951.

(17) WALLIS, G. C. Vitamins D and A in Alfalfa Hay. So. Dak. Agr. Expt. Sta. Circular 53. 1944.

(18) WALLIS, G. C., PALMER, L. S., AND GULLICKSON, T. W. The Relation of Vitamin D to the Calcium and Phosphorus Retention in Cattle as Shown by Balance Trials. J. Dairy Sci., **18**: 213–228. 1945.

THE VALUE OF OXYTOCIN FOR REDUCING FLUCTUATIONS IN MILK AND FAT YIELD[1]

H. P. ADAMS[2] AND N. N. ALLEN[3]

There is considerable daily variation in milk and fat production of dairy cows, even when as many as possible of the known causes are eliminated. This variation complicates the interpretation of experimental observations when the results are measured in milk yield or fat percentage. A factor which may contribute substantially to this variability is incomplete removal of the milk from the udder (3). Oxytocin, a hormone elaborated in the posterior pituitary, has been shown to effect a uniformly complete let-down of milk, even under adverse conditions (1, 2, 4, 5, 6).

The objective of this work was to determine the value of oxytocin for reducing daily variations in milk and fat production, when administered routinely at milking time to insure a uniformly complete milk-out. The work was carried out in two experiments. In experiment I, the effect of oxytocin upon daily fluctuations was studied under uniform feeding and environment. In experiment II, the effect of high fat intake upon milk and butterfat production was studied, first under a good milking routine; then under a similar routine, but with oxytocin administered at milking time to insure a complete milk-out.

Eight cows, including one Holstein, three Jerseys and four Guernseys, were used throughout both experiments. The milking routine followed was essentially that outlined by Petersen (7). The milk was weighed and tested by individual milkings, but the results were analyzed on a daily basis.

Experiment I. The Effect of Oxytocin on Daily Variations under Uniform Feeding and Milking Routine.

The intravenous injection of oxytocin has been shown to reduce the daily fluctuations in milk and fat in the case of cows known to "hold up" their milk (6). This experiment was carried out to determine whether oxytocin will reduce daily fluctuations in normal dairy cows which are milked by a good routine system. By reducing these fluctuations, oxytocin might be a valuable aid in comparing the effect of rations, environments, or treatments on production.

This experiment was designed as a reversal with two 10-day periods separated by a 2-day transition period. The eight cows were divided into two groups. Four cows received oxytocin during the first period, the other four during the second. One ml. of a preparation supplying 10 I.U. of oxytocin was injected into the jugular vein about 1 min. prior to the start of milking.

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RESULTS

The daily milk and fat production and fat test were analyzed by Snedecor's "homogeneity of variance" method (8) to determine whether oxytocin reduced the daily variation. The variation in milk yield was not reduced significantly in any of the eight cows. In fact, the daily variation was larger in four of eight cases when oxytocin was used. The daily variations in fat test and in fat yield were each reduced significantly in one of eight cases when oxytocin was used, and these reductions did not occur in the same animal. Three cows showed more variation in fat yield and two showed more variation in fat test when oxytocin was used.

These results indicate that oxytocin does not reduce significantly the daily variation in milk or fat yield or in fat test when the milking routine used induces a satisfactory natural let-down.

TABLE 1

Average daily milk and fat yield and average fat test of 8 cows over two 10-d. periods with and without oxytocin

Cow	Without oxytocin						With oxytocin					
	Milk	s	Fat	s	Fat	s	Milk	s	Fat	s	Fat	s
	(*lb.*)		(*lb.*)		(%)		(*lb.*)		(*lb.*)		(%)	
397	26.4	1.78	0.89	0.06	3.40	0.17	26.9	1.26	0.90	0.04	3.32	0.10
B63	35.1	2.01	1.42	0.09	4.04	0.51	38.5	3.25	1.55	0.17	4.04	0.57
B57	31.0	3.93	1.62	0.34	5.24	1.08	35.7	2.65	1.73	0.25	4.83	0.42
B58	38.4	3.21	1.51	0.20	3.93	0.34	42.5	2.29	1.69	0.06	3.97	0.17
588	12.2	1.02	0.57	0.06	4.66	0.42	16.7	1.75	0.76	0.11	4.52	0.37
543	15.0	1.12	0.86	1.0	5.75	0.45	20.2	1.01	1.13	0.09	5.56	0.33
575	27.0	1.10	1.20	0.05	4.44	0.14	31.2	1.14	1.29	0.08	4.14	0.24
705	20.2	2.95	1.20	0.33	5.95	1.20	19.5	3.37	1.23	0.17	6.31	0.52
Av.	25.7		1.16		4.68		28.9		1.28		4.59	

The data also were analyzed to determine whether oxytocin affected total milk, total fat or fat test over a 10-day period. Both total milk and total fat were increased significantly at the 1 per cent level when oxytocin was used (table 1). In all cases the total fat was increased, and in all cases except one the total milk was increased. The fat test was increased by oxytocin in two cases, decreased in five and remained unchanged in one. The average of all changes in test was too slight to be significant.

Experiment II. The Value of Oxytocin in Measuring the Effect of Level of Fat Intake.

It has been reported in previous literature that small amounts of fat added to the diets of dairy cattle increase the fat yield and fat test. This work was carried out to determine whether the use of oxytocin to secure a more uniformly complete milk-out might make the effects more clearly measurable by reducing daily fluctuations. This added sensitivity would make it possible to measure more accurately the effect of small differences in fat intake.

The experiment consisted of two reversal trials, each including two 10-day periods with a 2-day transition period. In the first trial, the effect of a difference in fat intake on fat yield and fat test was studied, milking the cows without oxytocin. During the second trial, immediately following the first, with the same group of cows, a similar comparison was made, using oxytocin. The fat content of the basic concentrate ration was approximately 4.2 per cent. One lb. of a mixture of equal parts of lard and butterfat (from goats' milk) was added to the basic daily grain ration to form the high-fat diet. The low-fat diet consisted of the basic grain ration plus 2.25 lb. of a mixture of equal parts of sugar and starch. This provided 2.25 lb. of carbohydrates to replace 1 lb. of fat, making the two diets comparable on an energy basis, but giving a difference of 1 lb. in the daily fat intake. When oxytocin was used in this experiment, 1 ml. of a preparation supplying 10 I.U. of oxytocin was injected about 1 min. prior to milking.

TABLE 2

Average daily production of cows fed a low-fat vs. a high-fat diet, milked with and without oxytocin

Cow	High fat			Low fat		
	Milk	Fat	Fat	Milk	Fat	Fat
	(*lb.*)	(*lb.*)	(%)	(*lb.*)	(*lb.*)	(%)
Without oxytocin						
397	21.7	0.89	4.12	25.0	0.84	3.38
B63	32.9	1.53	4.66	35.1	1.35	3.85
B57	33.2	1.87	5.64	31.6	1.60	5.07
B58	38.1	1.65	4.32	35.6	1.43	4.03
588	10.8	0.56	5.13	9.3	0.45	4.81
543	16.6	0.94	5.66	14.6	0.78	5.36
575	23.9	1.12	4.68	24.4	1.03	4.21
705	17.6	1.20	6.82	16.7	0.97	5.82
Av.	24.3*	1.22**	5.13***	24.0	1.06	4.57
With oxytocin						
397	19.0	0.88	4.60	22.6	0.87	3.85
B63	33.0	1.54	4.66	35.8	1.55	4.32
B57	34.8	1.88	5.38	34.3	1.77	5.15
B58	40.3	1.87	4.66	38.8	1.57	4.03
588	10.2	0.55	5.34	8.8	0.46	5.16
543	18.0	1.10	6.12	17.8	0.96	5.39
575	26.6	1.29	4.85	25.7	1.20	4.66
705	19.7	1.30	6.59	20.3	1.14	5.63
Av.	25.2*	1.30***	5.28**	25.5	1.19	4.77

* No significant difference.
** Significant difference at 1% level.
*** Significant difference at 2% level.

RESULTS

This experiment was analyzed to compare the effects of a high-fat and low-fat diet with and without oxytocin on total milk, total fat and average fat test. The totals for each cow are given in table 2. There was no significant difference in total milk between the two diets, either when oxytocin was used or when it was not used. There was a highly significant difference in total fat in favor of the

high-fat diet when no oxytocin was used. When oxytocin was used, the difference was significant at the 2 per cent level in favor of the high fat diet. Highly significant differences in the fat test in favor of the high fat diet were obtained with or without oxytocin, the difference being slightly greater when oxytocin was not used. From the above results it can be concluded that the accuracy of measurement of the effect of differences in fat intake is not increased by use of oxytocin as compared to a good milking routine which provides a satisfactory natural let-down.

SUMMARY

An experiment was conducted to determine whether the daily variation in milk and fat yield and fat test may be reduced by administering oxytocin at milking time to insure a complete milk-out. It was found that oxytocin did not reduce this variation when good milking practices were employed. Use of oxytocin caused a highly significant increase in total milk and fat without a significant change in the fat test.

In a second experiment, high- and low-fat diets were compared, first by normal milking procedures and then using oxytocin as an aid to complete milking. The total milk yield was not affected by the high fat intake. There was a significant increase in fat yield and fat test in favor of the high-fat diet. This was shown as clearly without the use of oxytocin as when it was used.

REFERENCES

(1) ELY, F., AND PETERSEN, W. E. Factors Involving the Ejection of Milk. J. Dairy Sci., **24**: 211–223. 1941.

(2) GAINES, W. L. A Contribution to the Physiology of Lactation. Am. J. Physiol., **38**: 285–312. 1915.

(3) GAINES, W. L., AND SANMANN, F. P. The Quantity of Milk Present in the Udder at Milking Time. Am. J. Physiol., **80**: 691–701. 1927.

(4) HAMMOND, J. The Effect of Pituitary Extract on the Secretion of Milk. Quart. J. Exptl. Physiol., **6**: 311–338. 1913.

(5) HILL, R. L., AND SIMPSON, S. The Effect of Pituitary Extract on Milk Secretion in the Goat. Quart. J. Exptl. Physiol., **8**: 103–111. 1914.

(6) KNODT, C. B., AND PETERSEN, W. E. The Effect of Complete Evacuation of the Mammary Gland by Pitocin upon Milk and Fat Production. J. Dairy Sci., **27**: 449–457. 1944.

(7) PETERSEN, W. E. The Action of the Mechanical Milker in Relation to Completeness of Milking and Udder Injury. J. Dairy Sci., **27**: 433–440. 1944.

(8) SNEDECOR, G. W. *Statistical Methods.* 4th ed. The Iowa State College Press, Ames. pp. 44, 249. 1946.

THE EFFECT OF REMOVAL OF RESIDUAL MILK BY USE OF OXYTOCIN UPON THE YIELD AND FAT CONTENT OF SUBSEQUENT MILKINGS[1]

H. P. ADAMS[2] AND N. N. ALLEN[3]

In work reported by the authors (1), it was found that milk and fat yield were increased significantly by use of oxytocin to secure more nearly complete milking out of cows. The milk and fat were increased to a similar extent, however, and the fat percentage was not altered significantly.

In view of the known fact that last milk or strippings is higher in fat content, it is interesting that the increased milk production due to securing the residual milk by use of oxytocin should not be accompanied by a higher fat test. This investigation was carried out in an attempt to secure an explanation for this relationship.

EXPERIMENTAL

Six cows, including two Jerseys and four Guernseys, were divided into two groups. A reversal plan was employed using the following procedure. From the average production for 3 days immediately prior to the experimental period, the anticipated milk production for each cow for each milking was estimated. This anticipated production was divided by three. Using this figure as a guide, an attempt was made to remove the milk from the cows of group 1 in three approximately equal successive portions. This was accomplished by milking into a machine suspended from scales. When the scale reading indicated that approximately one-third of the milk was secured, the teat cup and cover assembly was shifted to an empty milker pail, after which the second and third portions were taken in a similar manner. The udder was milked out for the third portion as completely as good machine-stripping procedure permitted. The first two portions thus secured were practically equal, the third varying slightly according to total milk obtained, but closely approximating one-third of the total milking.

The cows of group 2 were milked in a similar manner, except for the fact that 1 ml. of a preparation supplying 10 I.U. of oxytocin was injected into the jugular vein immediately following the milking out of the third portion of milk. The machine then was put on the cow again and the additional milk resulting from the administration of oxytocin was removed. Thus, in this group three normally secured portions of milk were obtained, as well as a fourth portion not secured by the normal let-down stimulus. At the end of a 5-day period, the two groups were reversed for the second period of 5 days, in addition to a 1-day transition period.

Each fraction of each individual milking was weighed and tested for butterfat.

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RESULTS AND DISCUSSION

In five of the six cows, the total daily milk production was increased, and in all cows the total fat was increased when oxytocin was used. In three cows the fat test was slightly higher and in the other three slightly lower when oxytocin was used. On the average, oxytocin increased daily milk production 1.34 lb. and the fat production 0.05 lb. per cow. The average test for all cows was slightly

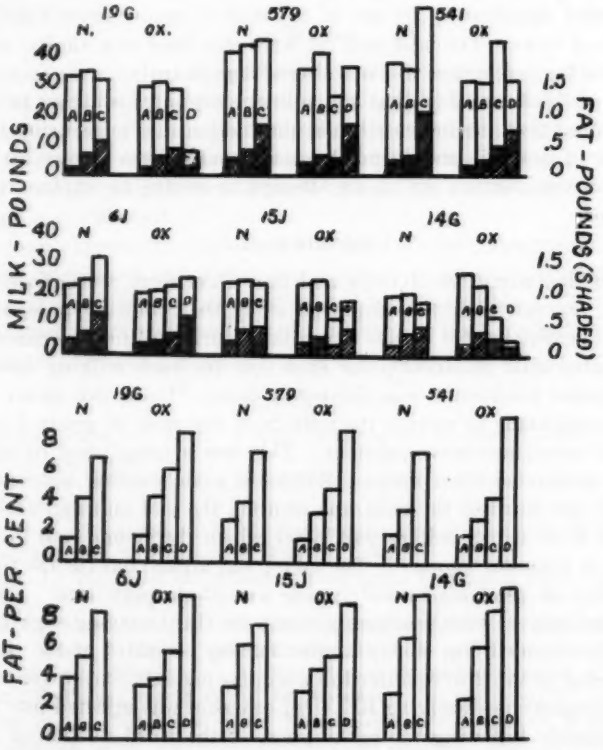

FIG. 1. Milk and fat yield (5-d. totals) and average fat percentage of milk from successive portions of milkings of individual cows. N = Normal milking. Ox = Oxytocin administered following normal milking. A, B and C = Successive normally removed portions. D = Additional milk secured after oxytocin administration.

but not significantly lower when oxytocin was used. The actual average tests were 5.19 per cent with oxytocin and 5.27 per cent without oxytocin.

The comparative amounts and fat content of these portions of milk are shown in fig. 1. In all cases except one, when oxytocin had been used at the previous milking, all of the three portions of milk obtained by normal milking tested lower than corresponding portions when not preceded by oxytocin milking. In this one case the first portion tested higher following the use of oxytocin (1.9 per cent

as compared to 1.7), but the second and third portions had a lower fat test. This cow was the one which gave less total milk when oxytocin was used.

In all cases the residual or "oxytocin" milk tested higher than any other portion. The highest 5-day average of oxytocin milk for any cow was 10.5 per cent. This is considerably lower than was reported by Ely and Petersen (3) and by Dodd and Foot (2). However, this is the average of a series of oxytocin milkings, while their figures are based on single milkings, preceded by normal milkings.

More total milk and fat were obtained when oxytocin was used, but the fat test was not changed significantly, even though the residual milk itself was of extremely high fat content.

For the normal milking period, the three successive portions of milk averaged 31.2, 32.4 and 36.4 per cent, respectively, of the total, which was reasonably close to the equal division desired. With the oxytocin milking, the averages were 27.4, 28.1 and 24.0 per cent, respectively, for the normally removed portions, and the additional milk secured with oxytocin was 20.5 per cent of the total. Approximately one-fifth of the milk present in the gland was not obtained by normal milking. However, the increased total milk secured by use of oxytocin was only about one-third of this amount, indicating that about two-thirds of this residual milk would have been secured at the following milking, had oxytocin not been used.

The fat yields were 16.2, 29.9 and 53.9 per cent, respectively, for the three portions when milked without oxytocin. With oxytocin the normally secured portions were 14.0, 22.8 and 25.7 per cent, with 37.5 per cent of the total secured with oxytocin. The increased total fat was equivalent to only 14 per cent of the oxytocin fat, indicating that 86 per cent of the fat in the oxytocin milk which was not secured by the normal milking routine would have appeared in the next milking had the hormone not been used for its removal. This indicates that almost one-third of the total fat obtained by normal milking already was elaborated in the gland at the time of the previous milking. This carry-over or delayed ejection of residual fat apparently is a normal occurrence when cows are milked by the usual procedures.

All three fractions of the normally obtained milk tested higher when oxytocin had not been used at the previous milking, but the difference was greatest in the third fraction. This indicates that some of the residual fat, if not removed by the use of oxytocin, is ejected in all three portions of the subsequent milking. It is residual only in the sense that it remains in the udder until the next milking, rather than being static in the alveoli. Some of it apparently is progressively washed into the duct system by the flow of freshly secreted milk as the udder fills up. This delayed ejection of the fat apparently is a normal occurrence under ordinary milking procedures.

SUMMARY

During 5-day control periods, using six cows, the milk was removed from the udders in three approximately equal portions and each portion was weighed and

tested. During experimental periods of the same length, the cows were milked in a similar manner, but in addition the residual milk was obtained by injection of oxytocin. It was found that normally drawn milk tested higher in all three portions when oxytocin had not been used at the previous milking than the corresponding portions when oxytocin had been used, indicating that the very high testing residual milk is secured with oxytocin at the expense of the fat content of the subsequent milking. This is interpreted as indicating that the residual milk is not static, but that most of it is secured under normal milking practices at the following milking. There is indication that a substantial part of the fat of milk secured under ordinary milking routine may regularly come from residual milk which was present in the gland at the time of the previous milking.

REFERENCES

(1) ADAMS, H. P., AND ALLEN, N. N. The Value of Oxytocin for Reducing Fluctuations in Milk and Fat Yield. J. Dairy Sci., **35**: 817–820. 1952.

(2) DODD, F. H., AND FOOT, A. S. Residual Milk. Agriculture (Engl.), **55**: 238–242. 1948.

(3) ELY, F., AND PETERSEN, W. E. Factors Involving the Ejection of Milk. J. Dairy Sci., **24**: 211–223. 1941.

THE THERMAL BEHAVIOR OF MILK FAT IN RAPIDLY COOLED CREAM

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Recognition of the phenomenon of a temperature rise of cream that has been cooled rapidly following pasteurization is perhaps as old as the practice of temperature observation itself. This rise in temperature continues over a period of 3 hr. or longer, but, characteristically, the greater part of it occurs during the first hour. It may vary from 1 to 3° C. or even higher, depending upon the fat content of the cream, rapidity of cooling and season of the year. This characteristic popularly and erroneously often has been ascribed to a slow attainment of temperature equilibrium between the fat globules and the plasma because of a low coefficient of heat transfer of the fat.

The phenomenon was observed by Chevreul (2), who reported that milk fat cooled from 40 to 26.5° C. started to crystallize. As crystallization progressed, the temperature of the fat rose to 32° C. Horn and Osol (3) observed a similar behavior at about the same temperature range when cacao butter was cooled and held in a Dewar flask. Other investigators, among them Rahn (6) and Richardson (7), reported observing a temperature rise when milk fat is cooled to a point at which crystallization takes place. These observations appear to have been restricted to fat in mass.

Rishoi and Sharp (9, 10) noted two phenomena which parallel the rise in temperature of cooled cream: (a) an increase in apparent specific heat and (b) a shrinkage in volume of the fat when held at constant temperature in the range below 25° C. These characteristics, being parallel in period of duration, temperature of occurrence and relative magnitude, logically might be conceived to be different manifestations of the same phenomenon, *i.e.*, crystal formation.

Change of phase from liquid to solid usually is accompanied by the liberation of heat and a reduction in volume. A corollary of the evolution of heat during crystallization is the heat of fusion, but because of the lag in crystallization the curve of the liberation of heat during crystallization and the curve of the heat of fusion are not identical.

This is a report of studies made to measure that part of the heat of solidification liberated per gram of fat in cream cooled rapidly to various temperatures in the 0 to 30° C. range.

PROCEDURE

Pint thermos bottles were used as containers. The thermometers used were calibrated in either 0.1 or 0.2° C. divisions and were checked against one certified by the U. S. Bureau of Standards. Estimates were made to the closest 0.05° C. The heat lost to or gained from the surroundings by the containers and their contents was found to be about 12 cal. per hour per degree centigrade difference.

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The heat required to raise the temperature of the container and thermometer stem was found to be 16 cal. per degree centigrade. In experiments in which calorie values are given, corrections have been made for heat gained from or lost to the surroundings for each time-temperature interval.

In order to account for the heat liberated by milk fat at various temperatures commonly encountered in dairy practice, cream at about 30° C. was mixed with water or with a 20 per cent brine solution. The desired temperature of the mixture was approached by varying the quantity of cream and the temperature of the cooling medium used. When the temperature of the mixture at zero time was calculated to be below 12° C., the 20 per cent salt brine solution was used for the cooling medium; above that temperature water was used. The temperatures recorded for zero time are calculated; all others are observed values. The temperature of the mixture for zero time was calculated by the following formula:

$$X = \frac{(Ww \times Tw \times Sw) + (Wc \times Tc \times Sc) + (Tw \times C)}{(Ww\ Sw) + (Wc\ Sc) + C}$$

In this expression X is the temperature of the water-cream or brine-cream mixture at zero time, Ww the weight of the water or brine solution used, Tw the temperature of the water or brine, Sw the specific heat of the water or brine, Wc the weight of the cream, Tc the temperature of the cream, Sc the specific heat of the cream and C the calories required to raise the temperature of the container and thermometer stem 1° C. The following values for specific heat have been used: milk fat, 0.475; skimmilk, 0.943; and 20 per cent sodium chloride, 0.807.

In the case in which a brine solution was used as a cooling medium, a correction was made for the heat of mixing of skimmilk and the brine. The heat of mixing was determined for 250 g. of a 20 per cent solution of brine and a variable quantity of skimmilk corresponding in general to the skimmilk content of the quantity of cream used. The values obtained were plotted, and the corrections for heat of mixing made on the basis of the skimmilk content of the cream used.

RESULTS

In preliminary trials in which cream was cooled in an ice water bath, Rishoi (8) found that the heat liberated during crystallization was about 6 cal. per gram of fat. However, studies (9) relating to the specific heat of milk fat indicated that much higher values should be obtained.

The data shown in table 1 are representative of results obtained using samples of winter cream obtained at the Sugar Creek Creamery, Danville, Ill., and at the Cass-Clay Cooperative Creamery, Moorhead, Minn. Values obtained on fat in summer cream were about 2 cal. per gram lower than those for winter cream at similar temperatures. The iodine numbers of the winter fat were 28.00, 28.55 and 28.24.

While about 95 per cent of the heat liberated at equilibrium by rapidly cooled milk fat in cream at temperatures between about 0 and 30° C. was liberated within about 3 hr., it is interesting to note that at temperatures in the 0 to 12° C. range, 50 per cent or more of the heat was liberated within a period of a few seconds. The rapid changes which occur in the fat of cream cooled to temperatures around

and below 30° C. emphasize the necessity for caution in interpreting data at those temperatures unless sufficient time has elapsed for equilibrium conditions to be established.

TABLE 1

Calories liberated per gram of fat in cream cooled rapidly from about 30° C. to various temperatures in the 0–30° C. range

Calculated temperature for zero time	0.08° C.	4.25° C.	11.75° C.	14.45° C.	17.45° C.	26.9° C.*	29.8° C.*
First observed temperature	2.35	6.5	12.5	14.8	17.7	26.9	29.8
Final equilibrium temperature	4.08	7.85	14.45	15.95	18.5	28.1	29.8
Lapsed time in minutes	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*
0.25	10.2	9.3	4.3	2.2	1.3	0	0
15	13.1	11.1	7.1	3.9	2.9	0.2	0
30	14.2	13.3	10.0	6.8	5.0	1.2	0
60	16.9	15.3	11.9	7.8	5.7	1.5	0
120	18.0	16.3	13.9	8.2	5.9	1.6	0
180	18.3	16.8	14.8	8.9	5.9	2.4	0
240	18.9	17.2	15.1	9.7	5.9	2.7	
300	18.7	17.7	15.5	10.2	.....	2.8	
360	18.7	18.1	15.1	10.4		2.8	
420		17.8		10.2			
% heat liberated at zero time compared with that at equilibrium	54.0	51.3	27.7	21.2	22.0		
Total heat liberated compared with that liberated at 0° C. (%)	100.0	95.7	82.0	55.0	31.0	12.5	0

* Cooled in an ice water bath.

DISCUSSION

The temperature at which complete solidification of milk fat is attained apparently has not been reported. This is one reason why full knowledge of the thermal behavior of milk fat is lacking in the temperature range within which milk fat changes from liquid-to-solid or solid-to-liquid. In the absence of exact data on the heat of fusion and the heat of solidification, some value may perhaps be assigned to other data relating to the thermal behavior of milk fat, within the temperature ranges commonly encountered in the processing of dairy products.

It was pointed out by Sommer (11) that milk fat may exist in a very large number of molecular combinations. A large number of these must contain unsaturated fatty acids which, in turn, as was shown by Oliver *et al.* (5), working with cottonseed oil, are associated with low heat of fusion when compared with fats more highly saturated. The nearest approach to determining the heat of fusion of milk fat to date appears to be the work of Jack and Brunner (4). They determined what they call "the heat of melting" of milk fat between −10

and 60° C., and report a value of 19.5 cal. per gram. This value has been considered as being "probably a low value" by Bailey (1). A higher value, determined experimentally, has not been reported. It is recognized that the values here reported represent neither the heat of fusion nor the heat of crystallization of milk fat, yet it may be pointed out that the heat liberated by milk fat in cream cooled rapidly from around 30° C. to about 0° C., 18.7 cal. per gram, is comparable to that required to melt the fat in cream cooled to – 10° C., 19.5 cal. per gram.

The heat of solidification liberated by milk fat and the time-temperature relationships involved have certain practical aspects. In an operation of cooling cream containing 40 per cent fat from 65 to 5° C., the heat liberated by the fat represents about 14 per cent of the total heat to be removed; in cooling plastic cream containing 80 per cent fat, it represents about 31.5 per cent; and in pure milk fat, nearly 40 per cent. While a large part of the heat of solidification may be removed during the cooling operation when vat cooling procedure is followed, a considerable temperature allowance is required when rapid surface cooling is practiced.

CONCLUSIONS

Representative values of heat of solidification liberated by milk fat in winter cream cooled rapidly from *ca.* 30° C. to various temperatures were found to be as follows:

° *C.*	*Calories*
0.08	18.7
4.25	17.8
11.75	15.1
14.45	10.2
17.45	5.95
26.90	2.80
29.80	0

REFERENCES

(1) BAILEY, A. E. *Melting and Solidification of Fats.* 238 pp. Interscience Publishing Co., New York. 1950.

(2) CHEVREUL, M. Faits pour servir a l'histoire du buerre de vache. Ann. de Chemie et de Physique. Serie I, **22**: 366–374. 1823.

(3) HORN, D. W., AND OSAL, A. Cacao Butter. Am. J. Pharm., **101**: 601–611. 1929.

(4) JACK, E. L., AND BRUNNER, J. R. The Relation between the Degree of Solidification of Fat in Cream and its Churning Time. I. Measurement of Degree of Solidification. J. Dairy Sci., **26**: 169–177. 1943.

(5) OLIVER, G. D., SINGLETON, W. S., TODD, S. S., BAILEY, A. E. Thermal Properties of Fats and Oils. II. Heat Capacity and Latent Heat of Cottonseed Oil. Oil and Soap, **21**: 297–300. 1944.

(6) RAHN, O. Der Erstarrungspunkt des Butterfettes. Milchwirtschaftliche Forschungen, **1**: 15–20. 1923.

(7) RICHARDSON, G. A. The "Transition Point" of Milk Fat. J. Dairy Sci., **19**: 749–752. 1936.

(8) RISHOI, A. H. Unpublished data. 1938.

(9) RISHOI, A. H., AND SHARP, P F. Specific Heat and the Physical State of the Fat in Cream. J. Dairy Sci., 21: 399–405. 1938.

(10) RISHOI, A. H., AND SHARP, P. F. Volume Changes of Fat in Cooled Cream Held at Constant Temperature. J. Dairy Sci., 21: 683–687. 1938.

(11) SOMMER, H. H. The Fat Emulsion in Milk from a Chemical Standpoint. Symposium on Milk Fat. Forty Sixth Annual Meeting of the American Dairy Science Assoc., U. of Tenn., Knoxville, Tenn. June, 1951.

THE AMERICAN DAIRY SCIENCE ASSOCIATION

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NATIONAL INTERCOLLEGIATE DAIRY CATTLE JUDGING CONTEST
NATIONAL DAIRY CATTLE CONGRESS—1952

WATERLOO, IOWA

TEAM RANK—ALL BREEDS

1.	Cornell Univ.	2009	6.	Iowa State College	1982
2.	Univ. of Maryland	2008	7.	Univ. of Wisconsin	1972
3.	Ohio State Univ.	1999	8.	Univ. of Nebraska	1969
4.	Univ. of Kentucky	1990	9.	Washington State College	1949
5.	Kansas State College	1985	10.	California Polytechnic Inst.	1948

HIGH INDIVIDUALS IN JUDGING ALL BREEDS

1.	Miss Barbara Riggs, Maryland	690	6.	Jim Schuh, Kansas	683
2.	Russell Schelkopf, Nebraska	689	6.	John Doherty, Washington (Tie)	683
3.	Herman Hensel, Cornell	686	8.	Billy Ridgway, Kentucky	677
3.	David Colwell, Ohio (Tie)	686	9.	Thomas Weller, Maryland	676
5.	John Wente, Kentucky	685	10.	John Wysong, Cornell	674

AYRSHIRE

Teams

1.	Iowa State College	408
2.	South Dakota College	397
3.	Kansas State College	396
4.	Univ. of Maryland	389
5.	Univ. of Nebraska	388
6.	Univ. of Wisconsin	387
6.	Calif. Polytechnic Inst.	387
8.	Texas Technological College	382
9.	Univ. of Kentucky	381
10.	Univ. of Illinois	380
10.	Univ. of Massachusetts	380

Individuals

1.	Jim Schuh, Kansas	145
2.	Russell Schelkopf, Nebraska	143
3.	William Norton, Wisconsin	141
4.	Richard Lomen, Iowa	140
5.	Cleo Tillotson, South Dakota	139
6.	Donald Smith, Illinois	138
6.	William Huffard, Maryland	138
8.	Ray Miller, Texas Tech.	137
9.	Barbara Riggs, Maryland	135
9.	Geo. Gransling, Wisconsin	135
9.	John Wysong, Cornell	135
9.	Merrill Richey, Iowa	135

BROWN SWISS

Teams

1.	Ohio State Univ.	431
2.	Univ. of Nebraska	423
3.	Univ. of Maryland	421
4.	Univ. of Wisconsin	420
5.	Univ. of Kentucky	412
6.	Texas A & M College	410
7.	Cornell Univ.	409
7.	Kansas State College	409
9.	Univ. of Illinois	406
10.	Oklahoma A & M College	400

Individuals

1.	Barbara Riggs, Maryland	147
1.	James Mellinger, Ohio	147
3.	Herman Hensel, Cornell	146
3.	Delbert Merritt, Nebraska	146
5.	Arden Alexander, Wisconsin	145
5.	Jennings Cunningham, W. Va.	145
7.	H. W. Haisler, Texas A & M	144
7.	John Gross, South Dakota	144
9.	Thomas Weller, Maryland	143
9.	John Doherty, Washington	143
9.	Russell Schelkopf, Nebraska	143

GUERNSEY

Teams

1.	Univ. of Nebraska	420
2.	Univ. of Wisconsin	403
3.	Univ. of Arkansas	395

Individuals

1.	Russell Schelkopf, Nebraska	149
2.	John Wysong, Cornell	144
2.	Joe Armstrong, Kansas	144

4.	Iowa State College	394
5.	Univ. of Tennessee	388
6.	Cornell Univ.	387
7.	Univ. of Maryland	384
8.	Kansas State College	381
9.	Washington State College	380
10.	Virginia Polytechnic Inst.	379

2.	Arden Alexander, Wisconsin	144
5.	R. G. Baldwin, Virginia	143
5.	Thomas Weller, Maryland	143
7.	Willard Berry, Washington	141
8.	Howell Wheaton, Purdue	140
9.	John Wente, Kentucky	139
9.	Richard Larson, Massachusetts	139

HOLSTEIN

Teams

1.	Univ. of Kentucky	418
2.	Cornell Univ.	417
3.	Iowa State College	414
4.	Washington State College	410
5.	South Dakota State College	402
6.	Univ. of Illinois	400
6.	Ohio State Univ.	400
8.	Univ. of Minnesota	399
9.	Kansas State College	398
10.	Univ. of Nebraska	396

Individuals

1.	Stewart Wolcott, Cornell	146
2.	Billy Ridgway, Kentucky	144
3.	Herman Hensel, Cornell	143
3.	Leslie Mathews, Iowa	143
5.	John Wente, Kentucky	140
6.	John Doherty, Washington	139
6.	Kenneth Getz, Illinois	139
6.	Ervin Akins, Ohio State	139
6.	Robert Barduson, Minnesota	139
10.	Barbara Riggs, Maryland	138
10.	Russell Schelkopf, Nebraska	138
10.	Jim Schuh, Kansas	138
10.	John Gross, South Dakota	138

JERSEY

Teams

1.	Ohio State Univ.	426
2.	Alabama Polytechnic Inst.	424
2.	Ontario Agri. College	424
4.	Calif. Polytechnic Inst.	423
5.	Univ. of Maryland	422
6.	Iowa State College	420
6.	Virginia Polytechnic Inst.	420
8.	Cornell Univ.	418
9.	South Dakota State College	417
10.	Univ. of Kentucky	415
10.	Univ. of Missouri	415

Individuals

1.	James Mellinger, Ohio	147
2.	David Colwell, Ohio	146
3.	Merrill Richey, Iowa	146
3.	Barbara Riggs, Maryland	146
5.	Duane Traylor, Kansas	144
5.	Billy Ridgway, Kentucky	144
5.	Virgil Ernest, Calif. Polytech. Inst.	144
8.	Arden Alexander, Wisconsin	143
8.	J. W. Brown, Ontario	143
8.	Robert Yates, Alabama	143

COLLEGIATE STUDENTS' INTERNATIONAL CONTEST IN JUDGING DAIRY PRODUCTS

CHICAGO, ILLINOIS, 1952

ALL PRODUCTS

	Teams		*Individuals*	
1.	Ohio State Univ.	10	Edward J. Haynes, Jr., Ohio State	35
2.	Cornell Univ.	15	John A. Tarr, Cornell	41
3.	Iowa State College	20	Bert W. Taylor, Ohio State	47
4.	Michigan State College	28	J. D. Wright, Mississippi State	52
5.	Mississippi State College	39	Thomas Williamson, Michigan State	59
6.	Univ. of Minnesota	40	Robert R. Farrar, Minnesota (Tie)	75
7.	Texas Technological College	41	Hugh O. Jaynes, Tennessee (Tie)	75
8.	Univ. of Massachusetts (Tie)	47	Ronald Mock, Washington State	84
8.	Univ. of Maryland (Tie)	47	Albert B. Bishop, Connecticut	90
10.	Kansas State College	49	Henry N. Charlap, Cornell	94

BUTTER

	Teams		*Individuals*	
1.	Cornell Univ.	42.84	Carl Zurborg, Iowa State	9.0
2.	Iowa State College	43.67	John A. Tarr, Cornell	11.5
3.	Ohio State Univ.	45.51	G. A. Noffsinger, Virginia Poly.	12.0
4.	Mississippi State College	45.84	John G. Burke, Vermont	12.58
5.	Michigan State College	47.78	Albert B. Bishop, Kentucky	12.84
6.	Univ. of Connecticut	50.35	J. D. Wright, Mississippi State	13.00
7.	Univ. of Illinois	51.17	Alvin F. Rich, Michigan State	13.50
8.	Virginia Polytechnic Institute	53.17	Hugh O. Jaynes, Tennessee	14.17
9.	Texas Technological College	54.18	Erwin W. Albrecht, Illinois	14.17
10.	Univ. of Maryland	54.34	Bill LaGrange, Iowa State	14.17

(All ties broken in favor of flavor)

CHEESE

	Teams		*Individuals*	
1.	Ohio State Univ.	100.35	Bert W. Taylor, Ohio State	30.50
2.	Univ. of Wisconsin	107.52	John A. Tarr, Cornell	30.67
3.	Iowa State College	107.68	Thomas Williamson, Michigan St.	31.00
4.	Michigan State College	112.48	Robert R. Farrar, Minnesota	31.84
5.	Univ. of Minnesota	113.68	Ronald Mock, Washington State	32.34
6.	Virginia Polytechnic Inst.	114.26	Ralph Cumbest, Mississippi State	32.67
7.	Cornell Univ.	115.19	Donald L. Brick, Wisconsin	32.84
8.	Washington State College	115.34	Edward J. Haynes, Jr., Ohio State	33.42
9.	Mississippi State College	116.44	Fred Axelgard, Utah State	34.01
10.	Utah State College	116.77	E. E. Seay, Virginia Polytech. Inst.	34.58

MILK

	Individuals		*Teams*	
1.	Robert R. Farrar, Minnesota	29.50	Univ. of Massachusetts	105.35
2.	Gwendolyn Judson, Massachusetts	30.01	Cornell Univ.	111.43
3.	Clifford J. Wengert, Cornell	31.25	Univ. of Minnesota	113.67
4.	Edward J. Haynes, Jr., Ohio State	35.17	Ohio State Univ.	114.84
5.	Albert B. Bishop, Connecticut	35.67	Kansas State College	118.01
6.	J. D. Wright, Mississippi State	35.93	Univ. of Kentucky	119.84

7.	Richard E. Brown, Kansas State	36.00	7.	Univ. of Illinois	122.84
8.	Richard F. Teegen, Iowa State	36.09	8.	Iowa State College	124.61
9.	Bert W. Taylor, Ohio State	36.17	9.	Univ. of New Hampshire	125.34
10.	Marilea Papalia, Massachusetts	36.34	10.	Univ. of Vermont	126.09

ICE CREAM

	Individuals			*Teams*	
1.	Charles Shearer, Penn State	16.17	1.	North Carolina State College	57.42
2.	Lester Finder, Wisconsin	17.50	2.	Ohio State Univ.	63.09
3.	Thomas Williamson, Michigan St.	17.67	3.	Univ. of Georgia	63.50
4.	D. Ronald Biggerstaff, N. C. State	18.17	4.	Michigan State College	66.67
5.	Hugh O. Jaynes, Tennessee	18.50	5.	Cornell Univ.	66.92
6.	Henry N. Charlap, Cornell	18.50	6.	Mississippi State College	67.61
7.	E. A. Day, Maryland	18.67	7.	Iowa State College	67.73
8.	Edward J. Jaynes, Jr., Ohio State	18.67	8.	Texas Technological College	67.99
9.	J. D. Wright Mississippi State	18.92	9.	Univ. of Massachusetts	68.67
10.	Gwendolyn Judson, Massachusetts	19.50	10.	Penn State College	70.52

(All ties broken in favor of flavor)

ERRATUM

On p. 659, the tenth line from the bottom of the page, the phrase "of a cationic type" should read "of an anionic type".

SUBJECT INDEX OF ORIGINAL ARTICLES[a]

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[a] Prepared by D. D. Deane.
* Abstract of paper read at Annual Meeting.

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[1] Prepared by D. D. Deane.

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